PATHOGENIC

MICRO-ORGANISMS

INCLUDING

BACTERIA AND PROTOZOA

A PRACTICAL MANUAL FOR STUDENTS, PHYSICIANS AND HEALTH OFFICERS

BY

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PREFACE TO SECOND EDITION.

THE past few years have added greatly to our knowledge of the two classes of pathogenic micro-organisms, bacteria and protozoa, representing respectively the lowest forms of the vegetable and animal kingdoms. The importance of the protozoa is now recognized, not only because of the diseases known to be caused by them, but also because of their possible connection with the exanthemata and syphilis. In view of these developments it is obviously essential that the student should be instructed in both fields. I believe that the inclusion of both subjects in a single volume offers the advantages of convenience, facility of instruction, and the presentation of comprehensive knowledge. In the present edition the section on the Protozoa, excepting malaria, was undertaken by Dr. A. W. Williams. The chapter on Malaria is from the pen of Mr. L. B. Goldhorn, Instructor in Pathology in the University and Bellevue Hospital Medical College. Needless to say, the revision of the part on Bacteria has been thorough, so that the book as a whole endeavors to reflect the latest knowledge in the whole domain. This edition, like the previous one, has been written for the student and physician rather than for the laboratory worker.

In the preparation of this edition I greatly missed the active assistance of Dr. Guerard, whose removal to a distant city prevented his co-operation, except in the reading of the proof. I am also indebted to Dr. Charles Bolduan, Assistant Bacteriologist in the Research Laboratory, for valuable help in preparing this work for press.

I take this opportunity of acknowledging much valuable help from the splendid work on the pathogenic micro-organisms edited by Kolle and Wassermann. Several photographs from their *Atlas* have been used.

W. H. P.

NEW YORK, 1905.

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PART I.

PRINCIPLES OF BACTERIOLOGY.

CHAPTER I.

INTRODUCTORY—HISTORICAL SKETCH.

ALTHOUGH most of the more important discoveries in bacteriology which place it on the footing of a science are of comparatively recent date, the foundations of its study were laid over two centuries ago. From the earliest times the history of bacteriology has been intimately associated with that of medicine. Indeed, it is only through the investigations into the life history of the vegetable and animal unicellular micro-organisms that our present knowledge of the etiology, course, and prevention of the infectious diseases has been acquired; and it is only by the practical application of the principles and methods of bacteriology that many diseases can be positively diagnosed or the problems which present themselves to the sanitarian be certainly solved. The prominent position which bacteriology already holds toward medicine is, moreover, daily increasing in importance. Original discoveries are constantly adding to the list of known germ diseases, and the outlook is favorable for eventually obtaining, through serums, through attenuated cultures, or through the toxic substances of the micro-organisms themselves, means for immunizing against, if not curing, many of the specific infections. Even at present bacterial products and protective serums are used successfully as preventives or curative agents in several of the most prevalent infectious diseases. An acquaintance, therefore, with the main facts concerning these micro-organisms is as necessary to the education of the modern physician as a knowledge of anatomy, pathology, chemistry, or any of the allied sciences.

But before entering into a detailed consideration of the subject it may be interesting and instructive to review briefly a few of the impor-

tant steps which led up to the development of the science, and upon which its foundation rests, in which we shall see that the results obtained were gained only through long and laborious research, and after many obstacles were met and overcome by indomitable perseverance and accurate observation and experiment.

The first probably authentic observations of living microscopic organisms of which there is any record are those of Kircher, in 1659. This original investigator demonstrated the presence in putrid meat, milk, vinegar, cheese, etc., of "minute living worms," but did not describe their form or character.

Not long after this, in 1675, Leeuwenhoeck observed in rain-water, putrid infusions, and in his own and other saliva and diarrhœal evacuations living, motile "animalculæ" of most minute dimensions, which he described and illustrated by drawings. Leeuwenhoeck practised the art of lens-grinding, in which he eventually became so proficient that he perfected a lens superior to any magnifying glass obtainable at that day, and with which he was enabled to see objects very much smaller than had ever been seen before. "With the greatest astonishment," he writes, "I observed distributed everywhere through the material which I was examining animalcules of the most microscopic size, which moved themselves about very energetically." The work of this observer is conspicuous for its purely objective character and absence of speculation; and his descriptions and illustrations are done with remarkable clearness and accuracy, considering the imperfect optical instruments at his command. It was not until many years later, however, that any attempt was made to define the characters of these minute organisms and to classify them systematically.

From the earliest investigations into the life history and properties of bacteria micro-organisms have been thought to play an important part in the causation of infectious diseases. Shortly after the first investigations into this subject the opinion was advanced that puerperal fever, measles, smallpox, typhus, pleurisy, epilepsy, gout, and many other diseases were due to contagion. In fact, so widespread became the belief in a causal relation of these minute organisms to disease that it soon amounted to a veritable craze, and all forms and kinds of diseases were said to be produced in this way, upon no other foundation than that these organisms had been found in the mouth and intestinal contents of men and animals, and in water.

Among those who were especially conspicuous at this time for their advanced views on the germ-theory of infectious diseases was Marcus Antonius Plenciz, a physician of Vienna. This acute observer, who published his views in 1762, maintained that not only were all infectious diseases caused by micro-organisms, but that the infective material could be nothing else than a living organism. On these grounds he endeavored to explain the variations in the period of incubation of the different infectious diseases. He also insisted that there were special germs for each infectious disease by which the specific disease was produced. Plenciz believed, moreover, that these organisms were

capable of multiplication in the body, and suggested the possibility of their being conveyed from place to place through the air.

These views, it is true, were largely speculative, and rested upon insufficient experiment; but they were so plausible, and the arguments put forward in their support were so logical and convincing, that they continued to gain ground, in spite of considerable opposition and ridicule, and in many instances the conclusions reached have since been proved to be correct. The fact that infectious diseases were of sudden occurrence, breaking out often in isolated places, and that they frequently remained clinging for long periods to certain localities, leaving others unaffected, was evidence that they were not produced by a gaseous infective agent. Moreover, the mode of infection, its unlimited development among large numbers of individuals, and gradual spread over wide areas—the incubation, course of, and resulting immunity in recovery from infectious diseases—all pointed to the probable cause being a living organism.

Among other distinguished men of the day whose observations exerted a most powerful influence upon the doctrine of infection may be mentioned Henle. His writings (*Pathological Investigations*, 1840, and *Text-book of Rational Pathology*, 1853), in which he described the relation of micro-organisms to infectious diseases, and defined the character and action of bacteria upon certain phases and symptoms of these affections, are remarkable for their clearness and precision.

But, meanwhile, the question which most interested these investigators into the cause of infectious diseases was: Whence are these micro-organisms derived which were supposed to produce them? Were they the result of spontaneous generation due to vegetative changes in the substances in which the organisms were found, or were they reproduced from similar pre-existing organisms—the so-called vitalistic theory? This question is intimately connected with the investigations into the origin and nature of fermentation and putrefaction.

Spallanzani in 1769 demonstrated that if putrescible infusions of organic matter were placed in hermetically sealed flasks and then boiled the liquids were sterilized; neither were living organisms found in the solutions, nor did they decompose; and the infusions remained unchanged for an indefinite period.

It was objected to these experiments that the high temperature to which the liquids had been subjected so altered them that spontaneous generation could no longer take place. This objection was met by Spallanzani by cracking one of the flasks and allowing air to enter, when living organisms and decomposition again appeared in the boiled infusions.

Another objection raised by the believers in spontaneous generation was that in excluding the oxygen of the air by hermetically sealing the flasks the essential condition for the development of fermentation, which required free admission of this gas, was interfered with. This objection was then met by Schulze, in 1836, by causing the air admitted to the boiled decomposable liquids to pass through strong sulphuric acid.

Air thus robbed of its living organisms did not produce decomposition; whereas when no such precautions were taken with the air admitted the boiled solutions quickly fell into putrefaction, and living organisms were found to be present.

Schwann in 1839 obtained similar results in another way; he deprived the air admitted to his boiled liquids of micro-organisms by passing it through a tube which was heated to a temperature high enough to destroy them. To this investigator is also due the credit of having discovered the specific cause—the yeast plant, or *saccharomyces cerevisiæ* —of alcoholic fermentation, the process by which sugar is decomposed into alcohol and carbonic acid.

Again, it was objected to these experiments that the heating of the air had perhaps brought about some chemical change which hindered the production of fermentation. Schroeder and von Dusch in 1854 then showed that by a simple process of filtration, which has since proved of inestimable value in bacteriological work, the air can be mechanically freed from germs. By placing in the mouth of the flask containing the boiled solutions a loose plug of cotton, through which the air could freely circulate, it was found that all suspended micro-organisms could be excluded, and that air passed through such a filter, whether hot or cold, did not cause fermentation of boiled infusions.

Similar results were obtained by Hoffmann in 1860, and by Chevreul and Pasteur in 1861, without a cotton filter, by drawing out the neck of the flask to a fine tube and turning it downward, leaving the mouth open. In this case the force of gravity prevents the suspended bacteria from ascending, and there is no current of air to carry them upward through the tube into the flask containing the boiled infusion.

Tyndall later showed (1876), by his well-known investigations upon the floating matters of the air, that in a closed chamber, in which the air is not disturbed by currents, all suspended particles settle to the bottom, the superincumbent air being optically pure, as is proved by passing a ray of light through it. He demonstrated that the presence of living organisms in decomposing fluids was always to be explained either by the pre-existence of similar living forms in the infusion or upon the walls of the vessel containing it, or by the infusion having been exposed to air which was contaminated with organisms.

These facts have since been practically confirmed on a large scale in the preservation of food by the process of sterilization. Indeed, there is scarcely any biological problem which has been so satisfactorily solved or in which such uniform results have been obtained; but all through the experiments of the earlier investigators irregularities were constantly appearing. Although in the large majority of cases it was found possible to keep boiled organic liquids sterile in flasks to which the oxygen of the air had free access, the question of spontaneous generation still remained unsettled, inasmuch as occasionally, even under the most careful precautions, decomposition did occur in such boiled liquids.

This fact was explained by Pasteur in 1860 by experiments showing

that the temperature of boiling water was not sufficient to destroy all living organisms, and that, especially in alkaline liquids, a higher temperature was required to ensure sterilization. He showed that at a temperature of 110° to 112° C., however, which he obtained by boiling under a pressure of one and one-half atmospheres, all living organisms were invariably killed.

Pasteur at a later date (1865) demonstrated that the organisms which resist the boiling temperature are, in fact, reproductive bodies, which are now known as *spores*.

In 1876 the development of spores was carefully investigated and explained by Ferdinand Cohn. He, and a little later Koch, showed that certain rod-shaped organisms possess the power of passing into a resting or spore stage under peculiar conditions of growth, and when in this stage they are much less susceptible to the injurious action of higher temperatures than when in their normal vegetative condition.

With this discovery the controversy of spontaneous generation, in so far as it related to identified bacteria, was finally settled. If these micro-organisms, some of them being capable of producing the more resistant spores, were present in the air, dust, soil, water, etc., it was easy enough to explain the irregularities in the previous experiments; nor was it any longer to be doubted that these bacteria, through their products, were the cause, not the effect, of fermentation and putrefaction, and that when organic substances were completely sterilized and protected against the entrance of living germs from without, no development of micro-organisms occurred in them.

Stimulated by the establishment of the fact, through Pasteur's investigations, that fermentation and putrefaction were due to the action of living organisms reproduced from similar pre-existing forms, and that each form of fermentation was due to a special micro-organism, the study of the causal relation of micro-organisms to disease was taken up with renewed vigor. Reference has already been made to the opinions and hypotheses of the earlier observers as to the microbic origin of infectious diseases. The first positive grounds, however, for this doctrine, founded upon actual experiment, were the investigations into the cause of certain infectious diseases in insects and plants. Thus, Bassi in 1837 demonstrated that a fatal infectious malady of the silkworm—*muscardine*—was due to a parasitic micro-organism. Pasteur later devoted several years' study to an exhaustive investigation into the same subject; and in like manner Tulasse in 1864 and Kühne in 1855 showed that certain specific affections in grains, the potato, etc., were due to the invasion of parasites.

Very soon after this it was demonstrated that micro-organisms were probably the cause of certain infectious diseases in man and the higher animals. Bacteriological research has always been of special interest to physicians. Many of the most distinguished physicians of the day, in the earlier history of the science, concerned themselves in these investigations, and the progress made during the past fifteen or twenty years has been largely due to their work. Davaine, a famous French

physician, has the honor of having first demonstrated the causal relation of a micro-organism to a specific infectious disease in man and animals. The anthrax bacillus was discovered in the blood of animals dying from this disease by Pollender in 1849 and by Davaine in 1850; but it was not until 1863 that the last-named observer demonstrated by inoculation experiments that the bacillus was the cause of anthrax.

The next discoveries made were those relating to wounds and the infections to which they are liable. Rindfleisch in 1866 and Waldeyer and von Recklinghausen in 1871 were the first to draw attention to the minute organisms occurring in the pyæmic processes resulting from infected wounds, and occasionally following typhoid fever. Further investigations were made in erysipelatous inflammations secondary to injury by Billroth, Fehleisen, and others, agreeing that in these conditions micro-organisms could almost always be detected in the lymph channels of the subcutaneous tissues.

The brilliant results obtained by Lister in 1863–1870, in the antiseptic treatment of wounds, to prevent or inhibit the action of infective organisms, exerted a powerful influence on the doctrine of bacterial infection, causing it to be recognized far and wide and gradually lessening the number of its opponents. Lister's methods were suggested to him by Pasteur's investigations on putrefaction.

In 1877 Weigert and Ehrlich recommended the use of the aniline dyes as staining agents in the microscopic examination of micro-organisms in cover-glass preparations.

In the year 1880 Pasteur published his discovery of the bacillus of fowl cholera and his investigations upon the attenuation of the virus of anthrax and of fowl cholera, and upon protective inoculation against these diseases. Laveran in the same year announced the discovery of parasitic bodies in the blood of persons sick with malarial fever, and thus started investigations upon the unicellular animal parasites.

In 1881 Koch made his fundamental researches upon pathogenic bacteria. He introduced solid culture media and the "plate method" for obtaining pure cultures, and showed how different organisms could be isolated, cultivated independently, and by inoculation of pure cultures into susceptible animals made, in many cases, to reproduce the specific disease of which they were the cause. To him more than any other are due the methods which have enabled us to prove absolutely in a broad sense the permanence of bacterial varieties. It was in the course of this work that the Abbe system of substage condensing apparatus was first used in bacteriology.

In 1882 Pasteur published his first communication upon rabies. A little later came the investigations of Loeffler and Roux upon the diphtheria bacillus and its toxins, and that of Kitasato upon tetanus. These researches paved the way for Behring's discovery of diphtheria antitoxin, which in its turn stimulated investigation upon the whole subject of immunity.

CHAPTER II.

GENERAL CHARACTERISTICS OF BACTERIA—RELATION TO OTHER MICRO-ORGANISMS—MORPHOLOGY AND STRUCTURE.

BACTERIA comprise the most important of the groups of micro-organisms which have in common the ability to invade the living tissues of animals and plants, and so become involved in the production of disease. The micro-organisms of some of the groups are undoubtedly animal structures, while others are clearly minute plants. Bacteria are such primitive forms that their differentiation is not marked, being related to both plants and animals, but their resemblance to plants seems to be so much closer that they are assigned to the vegetable kingdom. The bacteria are able to obtain their nourishment from much simpler chemical substances than the animal cells, yet they cannot use some of the substances which are assimilable by the green plants. Structurally and morphologically they are apparently extremely simple, although biologically they are very variable. Some bacteria are endowed with motility, others lack it. The majority are reproduced by transverse division, and in some respects they resemble the fungi; hence called by Naegeli "fission fungi, or schizomycetes." They are also somewhat allied to the lower algæ, especially in their ability to use simpler inorganic substances to build up higher compounds, but differ from them in not having chlorophyll. A few varieties of unicellular organisms resemble bacteria in all their known characteristics, except that they possess chlorophyll or substances similar to it. Others, still, which have no chlorophyll, are able, in the absence of light, to build up organic substances synthetically. The motile bacteria are closely related to the protozoa, some of which also invade animal tissues. The latter belong mostly to the animal kingdom and have a very wide distribution.

The bacteria are, therefore, a great class of micro-organisms which have relation on one or more sides to other classes. There are wonderful differences in the conditions of life and nutrition, which suit the different varieties. We meet with bacterial life between 0° and 75° C. Some live only in the tissues of men, others in animals, and by far the greater number in dead organic matter. For some free oxygen is necessary to life, for others it is a poison.

Bacteria may be defined as extremely minute, unicellular vegetable micro-organisms, which reproduce themselves with exceeding rapidity, usually by transverse division, and nourish themselves without the aid of chlorophyll. They have great powers of adapting themselves to

varied conditions. They have no nucleus, strictly speaking, but contain a substance which resembles nuclear material.

Those bacteria which depend entirely upon a living host for their existence are known as *strict parasites;* those which can lead a saprophytic existence, but which usually thrive only within the body of a living animal, are called *facultative parasites.* The *strict saprophytes,* which represent the large majority of all bacteria, while they destroy refuse are not only harmless to living organisms, but perform many exceedingly important functions in nature, such as the destruction of dead organic matter and its preparation for plant food through decomposition, putrefaction, and fermentation. The *parasites,* on the contrary, are harmful invaders of the body tissues, exciting by their growth and products many forms of inflammation and disease.

Relationship of Bacteria to Other Micro-organisms.—Bacteria are allied to moulds on the one hand, and to yeasts on the other. They have no differentiation into root, stem, and leaf. They resemble fungi, in that no sexual reproduction occurs, and that their mode of multiplication is usually by simple division. From such facts as these we may show their relations and build up a classification as follows:

Thallophyta (lower plants with no distinction between root and stem).

- Forms with chlorophyll (algæ, etc.).
- Forms without chlorophyll.
  - Multicellular: spores in differentiated spore-bearing organs. (The true fungi and moulds.)
  - Unicellular; spores frequently absent, spore-bearing cells little or not at all differentiated; no sexual reproduction.
    - The bacteria.
    - The yeasts (blastomycetes).

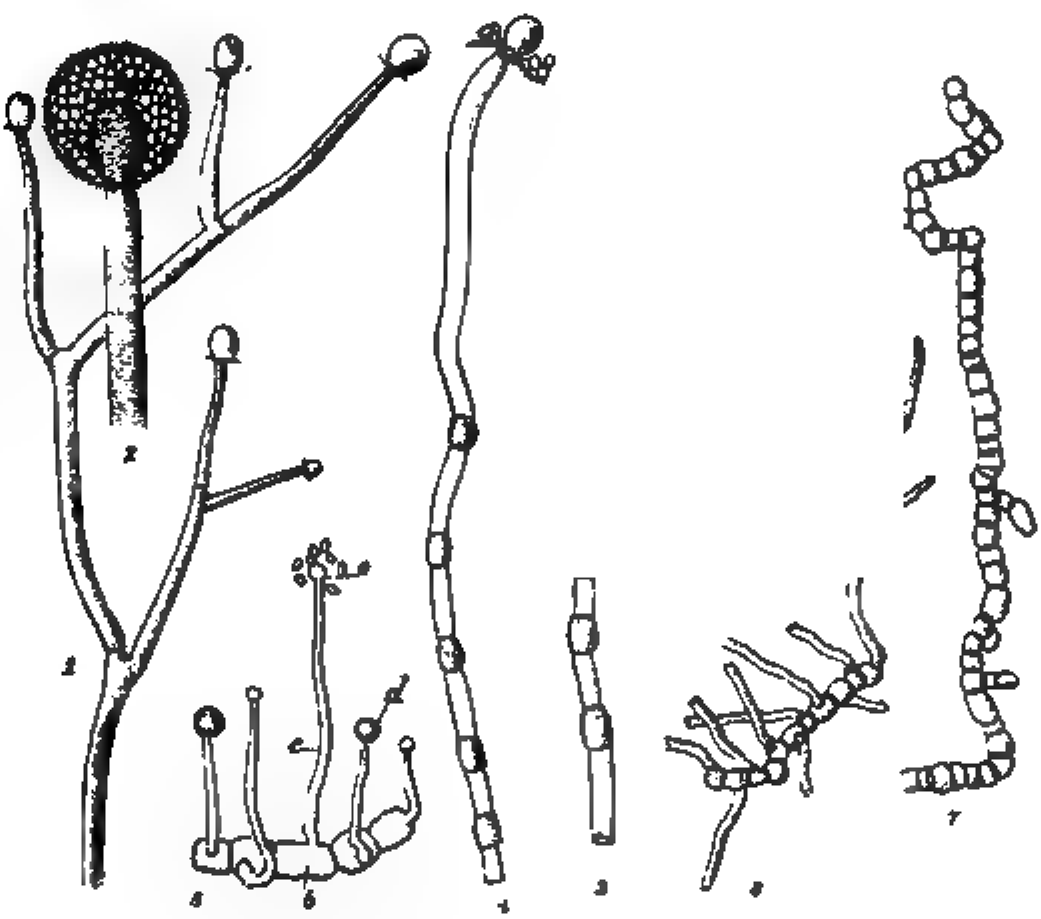
The bacteria may be subdivided into lower and higher forms. The lower forms are the more numerous and consist of minute unicellular masses of protoplasm. The largest of the forms met with in animals are 9μ, or micromillimetres (a $\mu = \frac{1}{25000}$ of an inch), long by less than 1μ thick. The smallest known bacteria measure 0.5μ long by 0.2μ thick, while others are invisible with any magnification which we now possess.

The higher forms (see streptothrix) show advance on the lower along two lines: (1) On the one hand they consist of filaments made up of simple elements, such as occur in the lower forms. These filaments may be more or less septate, may be provided with a sheath, and may show branching, either true or false. The minute structure of the elements comprising these filaments is analogous to that of the lower forms. Their size, however, is often somewhat greater. The lower forms sometimes occur in filaments, but here every member of the filament is independent, while in the higher forms there seems to be a certain interdependence among the individual elements. For instance,

growth may occur only at one end of a filament, the other forming an attachment to some fixed object. (2) The higher forms, moreover, present this further development that in certain cases some of the elements may be set apart for the reproduction of new individuals. The lower fungi have a still more complicated development (Fig. 1).

Morphology. BASIC FORMS OF THE LOWER BACTERIA.—The basic forms of the single bacterial cells are threefold—the sphere, the rod, and the segment of a spiral. Although under different conditions the type form of any one species may vary considerably, yet these three main divisions under similar conditions are permanent; and, so far as we know, it is never possible by any means to bring about changes in the

FIG. 1



1, branched filament carrying spores; 2, cross-section of spore highly magnified; 3 and 4, spore building; 5, developing and bursting spores; 6 and 7, branching; 8, sprouting spores. (After Tavel.)

organisms that will result in the conversion of the morphology of the members of one group into that of another—that is, micrococci always, under suitable conditions, produce micrococci, bacilli produce bacilli, and spirilla produce spirilla.

The form of the bacterial cells at their stage of complete development must be distinguished from that which they possess just after or just before they have divided. As the spherical cell develops preparatory to its division into two cells it becomes elongated and appears as a short oval rod; at the moment of its division, on the contrary, the transverse diameter of each of its two halves is greater than their long diameter. A short rod becomes in the same way, at the moment of its division, two cells, the long diameter of each of which may be

even a trifle less than its short diameter, and thus they appear on superficial examination as spheres.

As bacteria multiply the cells produced from the parent cell have a greater or less tendency to remain attached. This is on account of the slimy envelope which all bacteria have more or less developed. In some varieties this tendency is extremely slight, in others it is marked. This union may appear simply as an aggregation of separate bacteria or so close that the group appears as a single cell. According to the method of the cell division and the tenacity with which the cells hold together, we get different groupings of bacteria, which aid us in their differentiation and identification. Thus, whether the bacterial cell divides in one, two, or three planes, we get forms built in one, two, or three dimensions. If we group bacteria according to the characteristic form of the cells, and then subdivide them according to the manner of their division in reproduction and the tenacity with which the newly developed cells cling to one another, we will have the following varieties:

FIG. 2

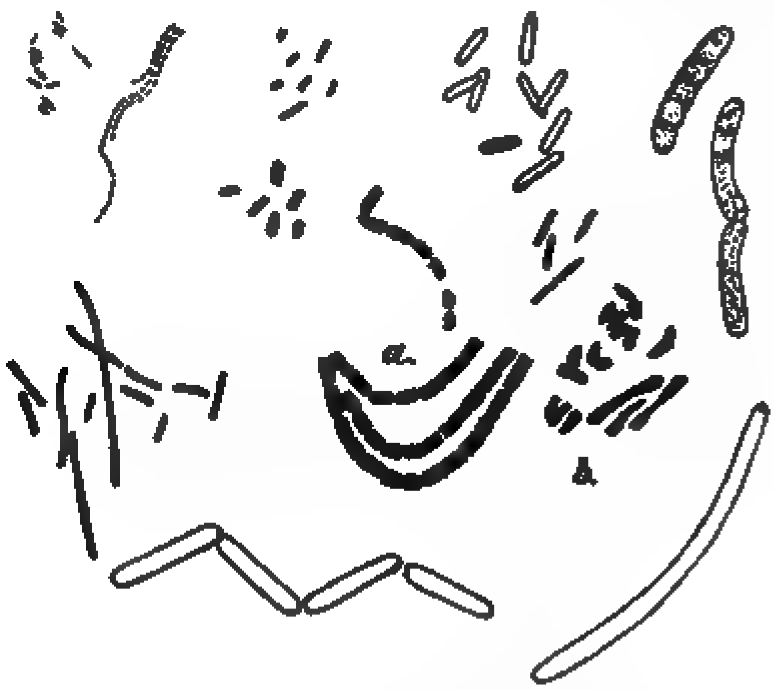
Varieties of spherical forms: *a*, tendency to lancet-shape; *b*, tendency to coffee-bean shape; *c*, in packets; *d*, in tetrads; *e*, in chains; *f*, in irregular masses. (After Flügge.)

1. SPHERICAL FORM, OR COCCUS (Fig. 2).—The size varies from about 0.3μ as minimum diameter to 3μ as maximum. The single elements are at the moment of their complete development, so far as we can determine, absolutely spherical; but when seen in the process of multiplication through division the form is seldom that of a true sphere. Here we have elongated or lancet-shaped forms, as frequently seen in the diplococcus of pneumonia, or the opposite, as in the diplococcus of gonorrhœa, where the cocci appear to be flattened against one another. Those cells which divide in one direction only and remain attached are found in pairs (diplococci) or in shorter or longer chains (streptococci). Those which divide in two directions, the one at right angles to the other, form bunches of four (tetrads). Those which divide in three directions and cling together form packets in cubes (sarcinæ). Those which apparently divide irregularly in any axis form irregularly shaped, grape-like bunches (staphylococci).

There are a considerable number of bacteria which appear to frequently assume spherical forms, or at least forms so like spheres that they cannot be differentiated from them, and yet under other conditions

they generate rod-like forms. These apparently spherical bacteria we can properly regard as short forms of bacilli, which, owing to the rapidity of division, are for the time being of the same size in both diameters. Under suitable conditions, however, the true rod-shape is always developed.

Fig. 3



Various forms of bacilli: *a*, bacilli with sides parallel to their long axis and with ends perpendicular; *b*, bacilli with sides swollen or narrowed, causing irregular forms. (After Flügge.)

2. Rod Form, or Bacillus.—The type of this group is the cylinder. The length of the fully developed cell is always longer than its breadth. The size of the cells of different varieties varies enormously, from a length of 30μ and a breadth of 4μ to a length of 0.2μ and a breadth of 0.1μ. The largest bacilli met with in disease do not, however, average over 3μ. In describing their forms bacilli are roughly classed as slender

Fig. 4 Fig. 5

Small bacilli, mostly in pairs.

when the ratio of the long to the transverse diameter is from 1:4 to 1:10, and as thick when the proportions of the long to the short diameter is approximately 1:2.

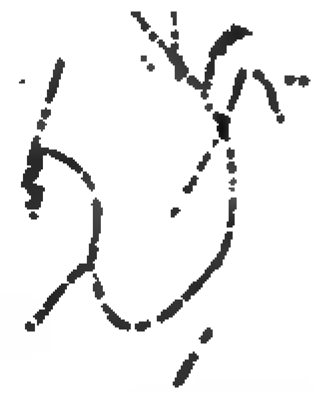
The characteristic form of the bacillus is one with a straight axis, uniform thickness throughout, and flat ends (Fig. 3 *a*, and Fig. 6); but

there are many exceptions to this typical form. Thus frequently the motile bacteria have rounded ends (Fig. 3); many of the more slender forms have the long axis bent; some few species, such as the diphtheria bacilli (Fig. 4), invariably produce many cells whose thickness is very unequal at different portions. Spore formation also causes an irregularity of the cell outline (Figs. 7, 17 and 18).

FIG. 6

Large bacilli in chains.

FIG. 7



Spores in centre of bacilli. (From Kolle and Wasserman.)

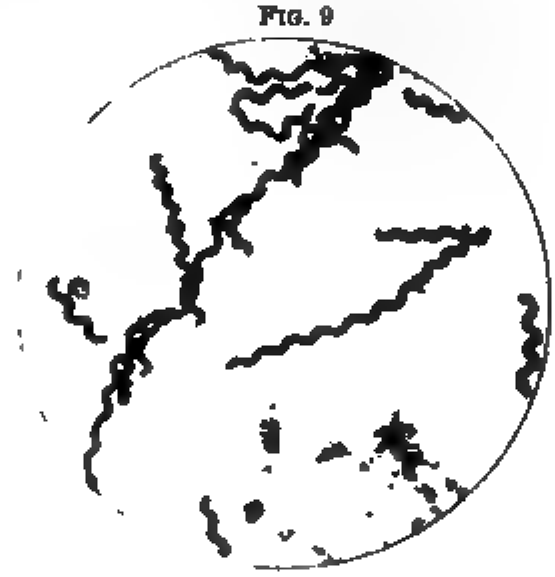
The bacilli except when they develop from spores or granules divide only in the plane perpendicular to their long axis. A classification, therefore, of bacilli according to their manner of grouping is much simpler than in the case of the cocci. We may thus have bacilli as isolated cells, as pairs, or as longer or shorter chains.

3. Spiral Form, or Spirillum.—The members of the third morphological group are spiral in shape, or rather segments of a spiral. Here, too, we have large and small, slender and thick spirals. The twisting of the long axis, which here lies in two planes, is the chief characteristic of this group of bacteria. Under normal conditions the twisting is equal throughout the entire length of the cell. The spirilla, like the bacilli, divide only in one direction. A single cell, a pair, or the union of two or more elements may thus present the appearance of a short segment of a spiral or a comma-shaped form, an S-shaped form, or a complete spiral or corkscrew-like form (Figs. 8, 9, 10 and 11).

FIG. 8

Medium-sized spirilla.

FIG. 9



Very large spirilla.

The determination of morphological characters for the description of bacteria should always be made from fully developed cultures; those which are too young may present, as already noted, immature forms, due to rapid multiplication, while in old cultures altered or degenerated forms may be observed.

When growth is obtained upon different media, variations, especially in size, may sometimes be observed. These differences should always

FIG. 10

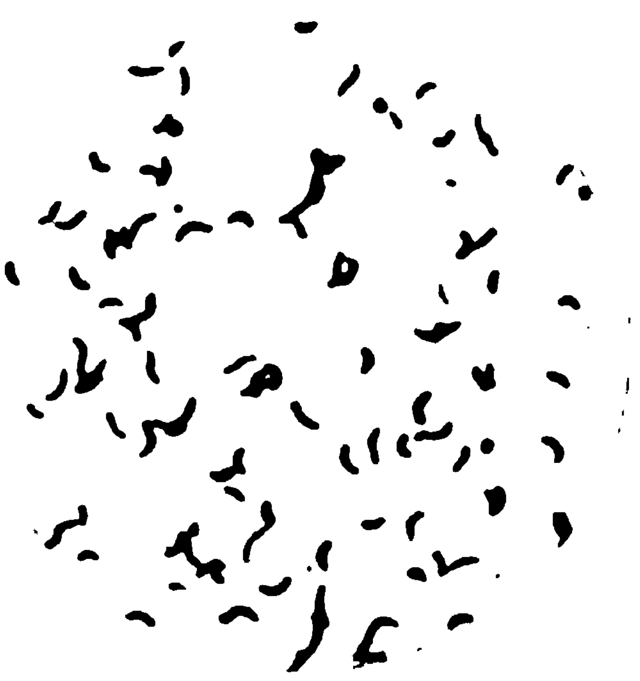
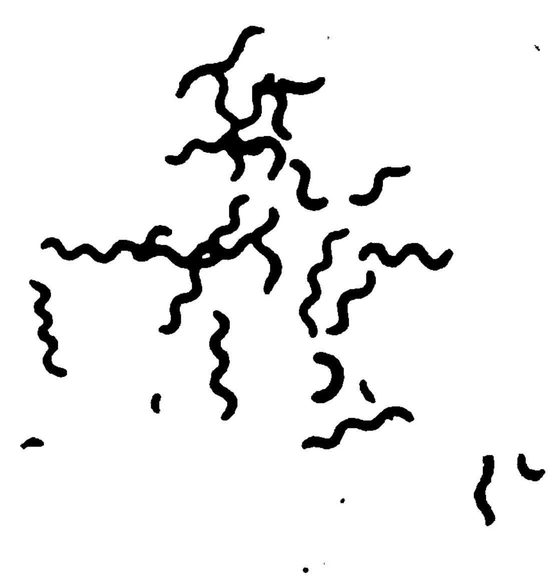


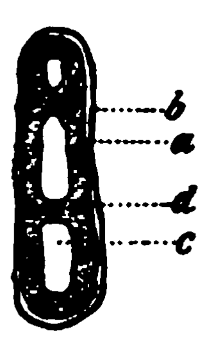
FIG. 11



be described, together with a note of the media upon which they were developed and a statement as to whether such variation is a marked feature of the species under consideration.

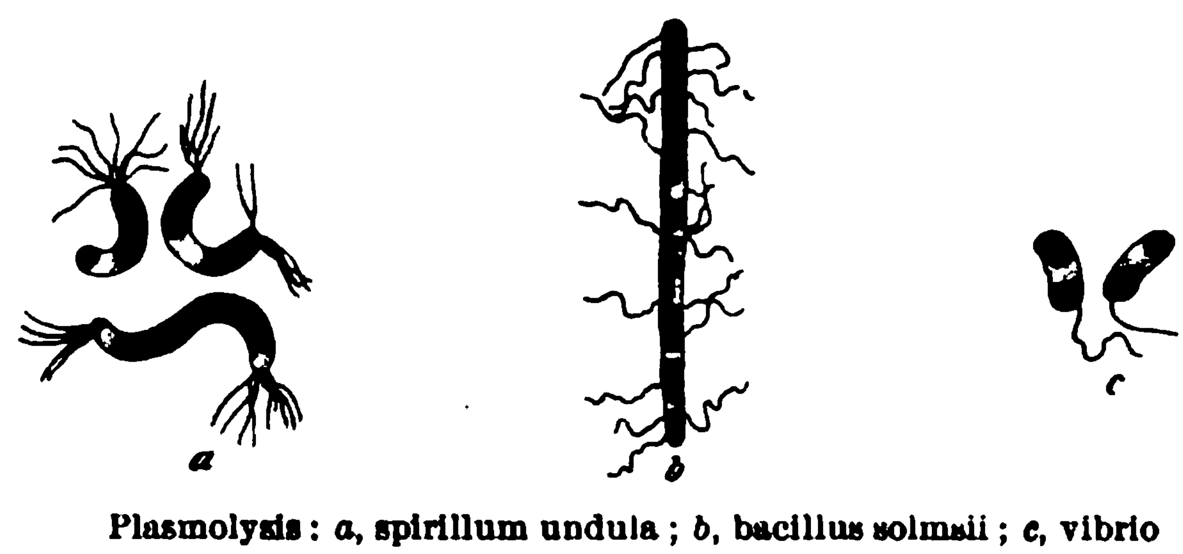
The conditions of temperature and of nutrition which favor growth are very various for different species, so that no fixed temperature, medium, or age of growth can be determined upon as applicable to all

FIG. 12



Structure of bacterial cells. (After Bütschli.)

FIG. 13



Plasmolysis: *a*, spirillum undula; *b*, bacillus solmsii; *c*, vibrio choleræ. (After A. Fischer.)

species. Morphological descriptions should always be accompanied by a definite statement of the age of the growth, the medium from which it was obtained, and the temperature at which it was developed.

Structure of Bacterial Cells.—When examined in water under the microscope bacteria appear merely as colorless refractile bodies with or without spores. It is only through using special stains that we are able to see more of their structure (Fig. 12). They are thus found to

have a rather indefinite cell membrane surrounding the central protoplasm. This, according to Zettnow, contains a nucleus, or at least the equivalent of the nucleus of the higher micro-organisms, lying within a network of protoplasma. The nuclear substance takes the chromatin stains and is often so abundant that the material holding it is covered up. The plasma substance divides into the entoplasma and the ectoplasma. The first is more or less intimately connected with the nuclear substance, and is especially collected at the ends of the long bacteria. This stains blue by the Romanowski method. The ectoplasma, or cell membrane, is a much more concentrated substance than the entoplasma, and remains unstained ordinarily, but by special stains appears as an external shell or flagella. The membrane surrounding some bacteria is very slightly developed; in others, as in tubercle bacilli, it is well developed. It is never a cellulose envelope like the higher plant cells, but by means of its chemical composition is differentiated from the inner plasma, as shown in plasmolysis. Thus, when bacteria are placed in 1 per cent. chloride of sodium the central body contracts and separates itself in places from the capsule, as shown in Fig 13.

THE CAPSULE.—The capsule consists of an inner tougher portion immediately surrounding the central body and which gradually passes into a thinner and more watery outer portion which is uncolored by ordinary staining method.

This is indicated by the greater apparent diameter of bacteria when stained with certain dyes beyond what they usually show. Certain bacteria, however, commonly known as *capsule bacteria*, as shown in Fig. 14, have the outer layers of the membrane so much thickened that the bacteria seem to be surrounded by a broad gelatinous envelope or capsule, which is distinguished by a diminished power of staining with the ordinary aniline dyes. The form of the capsule varies with different species (Fig. 12). The demonstration of this capsule is often of help in differentiating between certain bacteria—*e. g.*, some forms of the streptococcus and pneumococcus. A peculiarity of the capsule bacteria is that, except very rarely, they exhibit this envelope only when grown in the animal body or in special culture media, such as milk, blood serum, bronchial mucus, etc.; grown on nutrient gelatin agar or potato the capsule is only visible under very exceptional conditions, and then not distinctly.

FIG. 14

Pneumococcus illustrating capsule formation.

ORGANS OF MOTILITY.—The outer surface of bacteria, when occurring in the form of spheres, is almost always smooth and devoid of appendages; but the rods and spirals are frequently provided with fine, hair-like appendages, or *flagella,* which are their organs of motility (Figs. 15 and 16). These flagella, either singly or in numbers, are

sometimes distributed over the entire body of the cell, or they may form a tuft at one end of the rod, or only one polar flagellum is found. The polar flagella appear on the bacteria shortly before division. The flagella are believed to be formed of the ectoplasma, and probably have the property of protrusion and retraction. So far as we know, the flagella are the only means of locomotion possessed by the bacteria. They are not readily stained, special staining agents being required for this purpose. The envelope of the bacteria, which usually remains unstained with the ordinary dyes, then becomes colored and more distinctly visible than is commonly the case. Occasionally, however, some portion of the envelope remains unstained, when the flagella present the appearance of being detached from the body of the bacteria by a narrow zone. In cultures of richly flagellated bacteria peculiar pleated masses sometimes are observed, consisting of flagella which have been detached and then matted together. Bacteria may lose their power of producing flagella for a series of generations. Whether their power be permanently lost or not we do not know.

Fig. 15

Bacilli showing one polar flagellum.

Fig. 16

Bacilli showing multiple flagella.

Reproduction among the Lower Bacteria.—When a bacterial cell is placed in favorable surroundings for development it multiplies, as a rule, by simple division. When such development is in progress a single cell will be seen to elongate, in the case of spherical bacteria only slightly, and in the rod-shaped organisms considerably in one direction. Over the centre of the long axis thus formed will appear a slight indentation in the outer envelope of the cell; this indentation increases in extent until there exists eventually two individuals. As a rule, the cells separate from one another soon after division, but occasionally they remain together for a time, forming pairs and chains. Under certain conditions of nutrition long threads or filaments are formed, which, however, when put in contact with new food, break up into fragments. At times, when the culture media are exhausted or nearly so, the bacilli and spirilla will be found to go on dividing, with little or no increase

in length, and thus coccus-like forms result; but when these are given fresh food under suitable conditions they elongate and reproduce the usual shaped organisms. According to recent investigations on the subject of cell reproduction, the division of the cell starts from the protoplasmic layer, the central space being passively destroyed, and the outer envelope is only secondarily concerned in the process. This would indicate that the central space is not a true nucleus, otherwise the division of the nucleus should precede the cell division. The complete process of cell reproduction in most varieties occupies, under favorable conditions, about twenty to thirty minutes.

But although elongation in the greater diameter and transverse division is the rule for the majority of bacteria, there are certain groups, as the sarcinæ, for example, which divide more or less regularly in three directions. Instead of becoming separated from each other as single cells, the tendency then is for the segmentation to be incomplete, the cells remaining together in masses. The indentations upon these masses or cubes, which indicate the point of incomplete fission, give to these bundles of cells the appearance commonly ascribed to them—that of a bale of rags. As already said, division in two opposite directions results in the formation of a group of forms as tetrads. Division irregularly in all directions results in the production of clusters. The rod-shaped bacteria never divide longitudinally.

Reproduction among the Higher Bacteria.—Most of the higher bacteria consist of thread-like structures more or less septate and often surrounded by a sheath. The organism is frequently attached at one end to some object or to another individual. It grows to a certain length, and then at the free end certain cells called gonidia are cast off, from which new individuals are formed. The gonidia do not possess any special powers of resistance.

Spore formation must be distinguished from vegetative reproduction. This is the process by which the organisms are enabled to enter a stage in which they resist deleterious influences to a much higher degree than is possible for them in the growing or vegetative condition. It is true that in all cultures a certain proportion of the bacteria are more resistant than the average. No difference in protoplasm, however, has been noted in them. They are probably the youngest and most hardy or perhaps involution forms. The difference between these and the less resistant forms is not great. Some have believed that this resistance was due to certain bodies called *arthrospores*, or jointed spores, developed not within the cell, but as a sprout-like separation of one of its extremities. Recent researches into the formation of arthrospores have resulted in questioning their existence. There is, therefore, in the lower bacteria, only one kind of spores requiring special notice—viz., *endospores*. These are strongly refractile and glistening in appearance, oval or round in shape, and composed of concentrated protoplasm developed within the cell (Figs. 17 and 18). They are characterized by the power of resisting the injurious influences of heat, desiccation, and chemical disinfectants.

The production of endospores in the different species of bacteria, though not identical, is very similar. To observe the formation of spores in any species it is best to employ a streak culture on nutrient agar or a potato culture, which should be kept at the temperature nearest the optimum of the organism to be examined. At the end of twelve, eighteen, twenty-four, thirty, thirty-six hours, etc., specimens of the culture are observed first unstained in a hanging drop or agar block, and then, if round or oval, highly refractile bodies are seen, they should be stained for spores. A bacillus, as a rule, produces but one spore, and more than two have never been observed.

According to Fischer motile bacteria always come to a state of rest or immobility previous to spore formation. Several species first become elongated. The anthrax bacillus does this, and a description of the method of its production of spores may serve as an illustration of the process. In the beginning the protoplasm of the elongated filaments is homogeneous, but after a time it becomes turbid and finely granular.

FIG. 17

Unstained spores in slightly distended bacilli. (The spores are the light oval spaces in the heavily stained bacilli.)

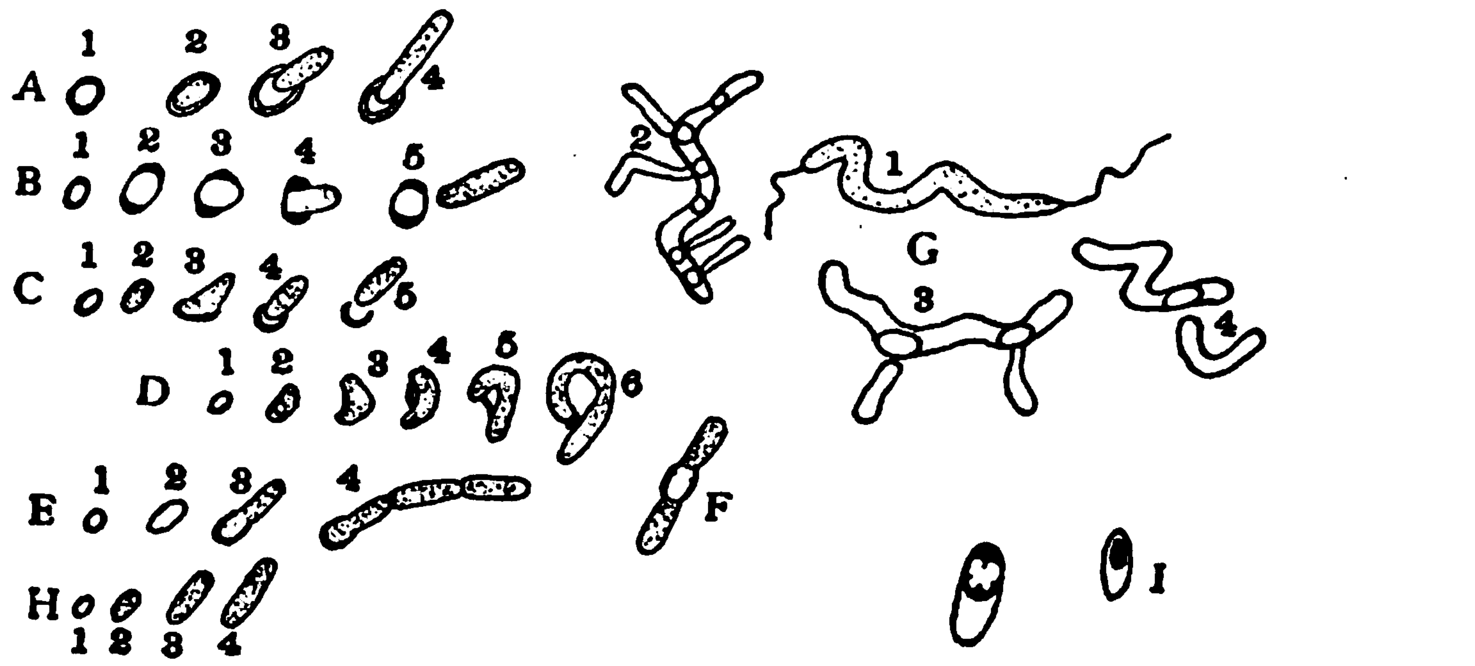
FIG. 18

Unstained spores in distended ends of bacilli.

These fine granules are then replaced by a smaller number of coarser granules, which are finally amalgamated into a spherical or oval refractile body. This is the spore. As soon as the process is completed there appears between two spores a delicate partition wall. For a time the spores are retained in a linear position by the cell membrane of the bacillus, but this is later dissolved or broken up and the spores are set free. Not all the cells that make the effort to form spores, as shown by the spherical bodies contained in them, bring these to maturity; indeed, many varieties, under certain cultural conditions, lose their property of forming spores. The following are the most important spore types: (*a*) the spore lying in the interior of single, short, undistended cells; (*b*) the spores lying in the interior of a chain of undistended cells (Fig. 7); (*c*) the spore lying at the extremity of a cell much enlarged at that end—the so-called "head spore" (Fig. 18); and (*d*) the spore lying in the interior of a cell very much enlarged in its central portion, giving it a spindle shape.

The *germination of spores* (Fig. 19) takes place as follows: By the absorption of water they become swollen and pale in color, losing their shining, refractile appearance. Later, a little protuberance is seen upon one side or at one extremity of the spore, and this rapidly grows out to

Fig. 19



Showing methods of spore germination: *A*, polar germination of B. butyricus (after Prazmowski); *B*, equatorial germination of B. subtilis (after Prazmowski); *C-D*, equatorial germination of B. tumescens and of Bact. carotorum (after A. Koch); *E-F*, polar germination of Bact. sessile (after L. Klein); *H*, germination by absorption of B. anthracis (after De Bary); *G*, endogermination in spirillum endoparagogicum (after Sorokin); *I-K*, spore formation in Bact. anthracis (after Migula).

form a rod which consists of soft-growing protoplasm enveloped in a membrane, which is formed of the endosporium or inner layer of the cellular envelope of the spore. The outer envelope, or exosporium, is cast off and may be seen in the vicinity of the newly formed rod. Sometimes the vegetative cell emerges from one extremity of the oval spore, and in other species the exosporium is ruptured and the bacillus emerges from the side.

Fig. 20

Involution forms from bacilli. (From Flügge.)

Involution Forms.—In old cultures of bacteria, in old abscesses, and in other places where the deleterious substances have developed and the foodstuffs have been largely used, the rapidity of division of the bacteria is lessened and there are frequently found very irregular or distorted forms, due to the abnormal development and division of the bacterial cells under the unfavorable conditions present. These are spoken of as *involution* or *degenerated* forms. If these deformed cells are placed under suitable conditions they produce again normally fashioned organisms. Cocci may produce irregular rods and bacilli threads. In old abscesses the involution forms may look entirely different from the well-developed cells.

Metachromatic Granules.—These appear in unstained bacteria as light-refracting, in stained preparations as deeply stained, granules.

They have a great affinity for dyes, and so stain readily and give up the stain with some difficulty. In certain bacteria, such as the diphtheria bacilli, they are especially well marked. Here they have diagnostic value. Besides the metachromatic granules there are others which take up stains with difficulty.

CHAPTER III.

THE CLASSIFICATION OF BACTERIA—PERMANENCE OF SPECIES —CHEMICAL COMPOSITION AND NUTRITION.

Genera and Species.—Bacteria have been classified in many different ways by different observers. As a rule the genera are based upon morphological characters and the species upon biochemical, physiological, or pathogenic properties. While the form, size, and method of division are the most permanent characteristics of bacteria, and so are naturally utilized for classification, nevertheless, in this basis of division there are decided difficulties. Thus, while the form and size of bacteria are fairly constant under the same conditions, we have already noticed that they may be quite different under diverse conditions. Another serious drawback for our purposes is that these morphological characteristics give no indication whatever of the relations of the bacteria to disease and fermentation—the very characteristics for which as physicians we study them. Other properties of bacteria which are fairly constant under uniform conditions are those of spore and capsule formation, motility, reaction to staining reagents, relation to temperature, to oxygen, and other food material, and, finally, their relation to fermentation and disease.

Taking any one of these properties of bacteria as a basis, we can classify them; but even here there will be groups which under certain conditions would be placed in one class and under others in another.

Thus, the power to produce spores may be totally lost or held in abeyance for a time.

The relations to oxygen may be gradually altered, so that an anaërobic species grows in the presence of oxygen. Parasitic bacteria may be so cultivated as to become saprophytic varieties, and those which have no power to grow in the living body may acquire pathogenic properties.

The possibility of making any thoroughly satisfactory classification is rendered still more difficult by the fact that many necessarily imperfect attempts have already been made, so that there is a great deal of confusion, which is steadily increased as new varieties are found or old ones reinvestigated and classified differently in the different systems.

As one of the more successful attempts to classify bacteria, the system devised by Migula is here given, simply as an example. The morphology of bacteria is used as the basis of the division:

	FAMILIES.
I. Cells globose in a free state, not elongating in any direction before division into 1, 2, or 3 planes	1. Coccaceæ.
II. Cells cylindrical, longer or shorter, and only dividing in one plane, and elongating to about twice the normal length before the division.	
a. Cells straight, rod-shaped, without sheath, non-motile, or motile by means of flagella	2. Bacteriaceæ.
b. Cells crooked, without sheath	3. Spirillaceæ.
c. Cells enclosed in a sheath	4. Chlamydobacteriaceæ.

1. *Coccaceæ.*

	GENERA.
Cells without organs of motion.	
a. Division in one plane	1. Streptococcus.
b. Division in two planes	2. Micrococcus.
c. Division in three planes	3. Sarcina.
Cells with organs of motion.	
a. Division in two planes	4. Planococcus.
b. Division in three planes	5. Planosarcina.

2. *Bacteriaceæ.*

Cells without organs of motion	1. Bacterium.
Cells with organs of motion (flagella).	
a. Flagella distributed over the whole body	2. Bacillus.
b. Flagella polar	3. Pseudomonas.

3. *Spirillaceæ.*

Cells rigid, not snake-like or flexuous.	
a. Cells without organs of motion	1. Spirosoma.
b. Cells with organs of motion (flagella).	
1. Cells with 1, very rarely 2 to 3 polar flagella	2. Microspira.
2. Cells with polar flagella-tufts	3. Spirillum.
Cells flexuous	4. Spirochæta.

4. *Chlamydobacteriaceæ* (higher bacteria).

Cell contents without granules of sulphur.	
a. Cell threads unbranched.	
I. Cell division always only in one plane	1. Streptothrix.
II. Cell division in three planes previous to the formation of gonidia.	
1. Cells surrounded by a very delicate, scarcely visible sheath (marine)	2. Phragmidiothrix.
2. Sheath clearly visible (in fresh water)	3. Crenothrix.
b. Cell threads branched	4. Cladothrix.
Cell contents containing sulphur granules	5. Thiothrix.

The above table makes changes in the designation of some of the most common bacteria, as in the restoration of the old title bacterium and the assigning to it of all non-motile, rod-shaped organisms, thus altering the name of some of the most common pathogenic bacteria from bacillus to bacterium. Other changes are seen in the spirilla. Any such scheme

is at times arbitrary in placing some varieties under one generic division and others closely allied in another. It has also the objection, already noted, that it is only one of several classifications already in use, and until an authoritative body agrees on some one it seems unwise in such a volume as this to change the usually employed names for others which are, perhaps, intrinsically better. Another important reason for waiting is that with the increase of our knowledge we are constantly changing the position of different bacteria. Thus, such a well-known germ as the tubercle bacillus is now found to produce, under certain conditions, long, thread-like branching forms; so that it ceases to be under the classification of Migula, either a bacillus or bacterium. We will, therefore, simply use in this book the older, less scientific nomenclature, of classing all rod forms as bacilli and all spiral forms as spirilla, and consider together, in so far as is practicable, certain groups of bacteria whose members are closely allied to each other in some one or more important directions.

Permanence of Bacterial Species.—When we come to study special varieties or groups of bacteria, such as the bacilli which produce typhoid fever, diphtheria, and tuberculosis, it is of great importance for us to determine, if possible, to what extent the peculiar characteristics which each of these groups of bacteria possess are permanent in the generations which develop from them.

We cannot believe that the multitude of bacterial varieties which now exist have always existed. The probability is very strong that with succeeding generations and changing conditions new bacterial varieties have developed with new characteristics.

From time to time the changing conditions under which life progressed probably exposed certain animals to the invasion of varieties which never before had gained access to them. If the bacteria found some means of transmission to other animals equally susceptible, a parasitic species became established which at first, perhaps, found conditions only occasionally favorable to it. Thus in some such way a multitude of bacterial groups arose, some of which accustomed themselves to the conditions present in living plants, others to those in fishes, others to those in birds, and others still to those in man and the higher animals.

These are, however, theories. What has been actually observed in the few years during which bacteria have been studied? In this short time the pathogenic species as observed in disease have remained practically unaltered. The diphtheria bacilli are the same to-day as when Loeffler discovered them in 1884, and the disease itself is evidently the same as history shows it to have been before the time of Christ. The same permanence of disease type is true for tuberculosis, smallpox, hydrophobia, leprosy, etc. Under practically unchanged conditions, therefore, as exist in the bodies of men, bacteria which have once become established as parasites continue, so long as they remain, to retain their peculiar (specific) characteristics. Whether new disease varieties, such as the influenza bacillus, are coming into existence from time to time, is,

of course, a possibility, but not a certainty. The one thing we can probably safely assert is that there is no probability that any saprophytic variety now existing can, under any possibility, develop into the now recognized varieties of pathogenic bacteria. It is almost impossible to conceive that any such variety should develop parasitic tendencies, under exactly the same circumstances as those varieties which now produce disease.

The fact that the chief pathogenic varieties of bacteria, which excite disease in man, seem to have retained for centuries their characteristics, in no way proves that when placed under different conditions they would remain stable. As already stated the characteristics of bacteria can be radically altered, but they then lose those special traits which enable them to excite disease and so cease to exist as parasites and if they have lost the capacity to live as saprophytes they cease to exist altogether.

Attenuation.—As just stated it is established that the great majority of parasitic bacteria can be so altered by being grown outside the body, and especially by being subjected to unfavorable conditions, that they, while morphologically the same, lose their power of developing in the body and of producing specific poisons. When either or both of these properties are partially destroyed they can usually be redeveloped; but when absolutely lost they probably cannot be.

The recovery of toxin production is brought about by developing the micro-organism for a considerable length of time under the conditions best suited for it. The recovery of the ability to grow in the body of any animal species is brought about by causing the germ to develop in a series of animals of the same species whose resistance has been overcome by reducing their vitality through poisons, heat, cold, etc. Another method is to accustom the micro-organism to the animal's body by letting it remain surrounded by the animal fluids as it rests in a pervious capsule in the peritoneal cavity, or by growing it in unheated fresh serum or blood media.

Chemical Composition of Bacteria.—Qualitatively considered, the bodies of bacteria consist largely of water, salts, fats, and albuminous substances. There are also present, in smaller quantities, extractive substances soluble in alcohol and in ether. Special varieties contain unusual substances, as wax and cellulose in tubercle bacilli. Bacteria possess the capacity in a high degree of accommodating their chemical composition to the variety of soil in which they are growing. The same variety of bacteria thus varies greatly in the quantitative estimation of its chemical constituents. Each variety yields proteid substances peculiar to itself, as shown in the effects produced by animal inoculation. At present we know but little concerning the differentiation of these specific substances. This subject will be taken up in detail under bacterial toxins, etc. According to Cramer many bacteria contain amyloid substances which give a blue reaction with iodine. True cellulose has been found in some bacteria, and also large quantities of a gelatinous carbohydrate similar to hemicellulose have been obtained.

Nuclein is found frequently. The nuclein bases—xanthin, guanin, and adenin—have been obtained in considerable amounts. There is a group of bacteria which contain sulphur—viz., the *beggiatoa*—and another group, the *cladothrix*, is capable of separating ferric oxide from water containing iron.

Some light has been thrown upon the chemical composition of bacteria, quantitatively, by the studies of Cramer, though so far only a few species have been thoroughly investigated. The percentage of water contained in bacteria grown on solid culture media, as well as the amount of ash, depends largely on the composition of the media. Thus the bacillus prodigiosus when grown on potato contains 21.5 per cent. of dry residue and 2.7 per cent. of ash; when cultivated on turnips it contains 12.6 per cent. of dry residue and 1.3 per cent. of ash. Besides the concentration of the culture, its temperature and age also influence the amount of residue and ash produced. The residue varies, moreover, in its composition in the same species under the influence of the culture media employed.

It appears that an additional quantity of peptone in the culture media tends to increase the percentage of nitrogenous matter in the bacillus, while the addition of glucose decreases it.

Chemical Substances Necessary for the Nutrition of Bacteria.—The majority of bacteria are easily cultivated, but there are some for which, with our present knowledge, we are unable to produce conditions suited for their growth.

All bacterial culture media must contain an abundance of water; salts are also indispensable, and there must be organic material as a source of carbon and nitrogen. The greater number of important bacteria and all the pathogenic species thrive best in media containing albuminoid substances and of a slightly alkaline reaction to litmus. The demands of bacteria in the composition of the culture media vary considerably. There are some species of water bacteria, for instance, which require so little organic material that they will grow in water that has been twice distilled. A certain species will grow abundantly in water containing ammonium carbonate in solution and no other source of carbon and nitrogen. This shows the power of some bacteria of producing cell substance from the simplest materials—a power which belongs to the higher plants which obtain their nourishment from the air through their chlorophyll and the assistance of sunlight. Few bacteria, however, of any importance in medicine are so easily satisfied, though there are many species which are able to develop without the presence of albumin and in comparatively simple culture media, such as the culture liquid proposed by Uschinsky, or the simpler one of Voges and Fraenkel, which consists of water, 1000; sodium chloride, 5; neutral sodium phosphate, 2; ammonium acetate, 6; and asparagin, 4. In these media many bacteria grow well.

When we consider in detail the source of the more important chemical ingredients of bacteria we find that their nitrogen is most readily obtained from diffusible albuminoid material and less easily from ammonium

compounds. Their carbon they derive from albumin, peptone, sugar, and other allied carbohydrates; glycerin, fats, and other organic substances. It is an interesting fact that even compounds which in considerable concentration are extremely poisonous, can, when in sufficient dilution, provide the necessary carbon; thus some derive it from carbolic acid in very dilute solutions.

The value of substances as a source of nutrition is often influenced by the presence of other materials, as, for instance, the value of asparagin is increased by the presence of sugars. Further, materials from which nitrogen and carbon cannot be directly obtained still become assimilable after being subjected to the influence of bacterial ferments. The profound and diverse changes produced by the different ferments make it almost impossible to establish, except in the most general way, the nutritive value of any mixture for a large number of bacteria through a simple knowledge of its chemical composition. The special culture media, such as bouillon, blood serum, etc., for the development of bacteria, will be dealt with in a later chapter.

Relation of Bacteria to Oxygen.—The majority absolutely require oxygen for their growth, but a considerable minority fail to grow unless it is excluded. A knowledge of this latter fact we owe to Pasteur, who divided bacteria into aërobic and anaërobic. Between these two groups we have those that can grow either with or without the access of oxygen.

Some at least of the strict anaërobic bacteria require for the full development of their life functions the presence of fermentable substances, such as sugars, from which they obtain oxygen. Among bacteria can be found all gradations between those bacteria which develop only in the presence of oxygen to those which develop only in its absence. In so far as for any variety the amount of oxygen present is unfavorable there will be more or less restriction in some of the life processes of these bacteria, such as pigment and toxin production, spore formation, etc. It has also been found that some, at least, of the aërobic bacteria can be accustomed to grow without oxygen and that some of the anaërobics can be accustomed to grow with it Free oxygen kills the vegetable forms of the anaërobic bacteria in a few hours, but hardly injures the spores.

Sulphur and phosphorus are two important foodstuffs required by bacteria. Either calcium or magnesium and sodium or potassium are also usually required for bacterial growth. Iron is demanded by but few varieties, among which is the influenza bacillus.

When we consider the more complex culture media, either those naturally existing, such as blood serum, or those created by us for the cultivation of bacteria, we find, beyond the necessary amount of soluble foodstuffs, that the relative proportion of each form and the total concentration are of great importance. It is, nevertheless, true that very wide differences can exist with but slight effect upon the development of many bacteria, the development of the bacteria usually ceasing through the accumulation of deleterious substances in the culture media rather than through food exhaustion.

Influence of Reaction of Media upon Growth.—The reaction of the nutritive media is of very great importance. Most bacteria grow best in those that are slightly alkaline or neutral to litmus. Only a few varieties require an acid medium, and none of these belong to the parasitic bacteria. An amount of acid or alkali insufficient to prevent the development of bacteria may still suffice to rob them of some of their most important functions, such as the production of poison. The different effect upon closely allied varieties of bacteria of a slight excess of acid or alkali is sometimes made use of in separating those which may be closely allied in many other respects.

Influence of One Species upon the Growth of Others.—The influence of one species upon the growth of another, either when the bacteria grow together or follow one another, is very marked. The development of one variety of bacteria in a medium causes that substance, in the majority of instances, to become less suitable for the growth of other bacteria. This is due partly to the impoverishment of the foodstuff, but more to the production of chemical substances or enzymes, which are antagonistic not only to the growth of the bacteria producing them, but to many other varieties also; less frequently the changes produced by one variety of bacteria in the foodstuff are favorable for some other form. The pneumococcus which usually develops very tiny delicate colonies upon nutrient agar, grows as luxuriant succulent colonies when grown with certain bacilli.

Temperature Suitable for Growth.—For the growth of bacteria a suitable temperature is absolutely requisite. For different varieties the most favorable temperature varies, but for all a range of about 2.5° C. above or below this most favorable point covers the limits for their most vigorous growth. Few bacteria grow well under 10° C. and few over 40° C.; 2° C. is about the lowest temperature that any bacteria have been found to grow and 70° C. the highest.

In many instances the temperature of the soil in which the bacteria are deposited is the controlling factor in deciding whether growth will or will not take place. Thus, nearly all parasitic bacteria require a temperature near that of the body for their development, while many saprophytic bacteria can grow only at much lower temperatures. Bacteria when exposed to lower temperature than suffices for their growth, while having their activities decreased, are not otherwise injured unless actually frozen; while exposure to higher temperatures than allows of growth destroys the life of the bacteria. The relations of the temperature to bacterial life and death will be dealt with more fully in the next chapter.

CHAPTER IV.

EFFECT OF TEMPERATURE UPON THE GROWTH OF BACTERIA.

In judging the effect on bacteria of heat as well as other agents we have to note the important fact that different species are differently influenced by the same substance. Some bacteria live under conditions which would destroy others, and they vary among themselves in their powers of resistance to influences which are deleterious to all.

Further, any species of bacteria will resist better when under favorable conditions than under unfavorable ones. Bacteria also in recent cultures withstand injury better than those in old cultures, so long as they have not entered into the spore form. According to the amount of injury they have suffered, bacteria may be inhibited in some of their functions or they may be totally destroyed.

Bacteria Divided According to the Temperatures at which they Grow Best.—Some form of bacterial life is possible within the limits of 0° and 70° C. There are some species, however, which grow at the lower and others at the upper limit of these temperatures. The maximum and minimum temperature for each individual species lies from 10° to 30° C. apart. Bacteria have been classified according to the temperatures at which they develop, as follows:

Psychrophilic Bacteria.—Minimum at 0° C., optimum at 15° to 20° C., maximum at about 30° C. To this class belong many of the water bacteria, such as the phosphorescent bacteria in sea-water.

Mesophilic Bacteria.—Minimum at 5° to 25° C., optimum at 37° C., maximum at about 43° C. To this class belong all pathogenic bacteria.

Thermophilic Bacteria.—Minimum at 25° to 45° C., optimum at 50° to 55° C., maximum at 60° to 70° C. This class includes a number of soil bacteria which are almost exclusively spore-bearing bacilli. They are also found widely distributed in feces.

By carefully elevating or reducing the temperature the limits within which a variety of bacteria will grow can be altered. Thus, the anthrax bacillus was gradually made to accommodate itself to a temperature of 42° C., and pigeons, which are comparatively immune to anthrax, partly on account of their high body temperature (42° C.), when inoculated with this anthrax succumbed to the infection. Another culture accustomed to a temperature of 12° C. killed frogs kept at 12° C. We have cultivated a very virulent diphtheria bacillus so that it will grow at 43° C. and produce strong toxin.

Effect of Low Temperature.—Bacterial growth is retarded by temperatures lower than their optimum, although the bacteria are not otherwise

injured. Indeed, it is the usual custom in laboratories to preserve bacteria which die readily (such as streptococci) by keeping them in the refrigerator at about 4° to 6° C., after cultivation for two days at 30° C., as a means for retaining their vitality without repeated transplantation. Temperatures even far under 0° C. are only slowly injurious to bacteria, different species being affected with varying rapidity. This has been demonstrated by numerous experiments in which they have been exposed for hours in a refrigerating mixture at —18° C. They have even been subjected to a temperature of —175° C. by immersing them in liquid air kept in an open tube for two hours, and 15 to 80 per cent. were found to still grow when placed in favorable conditions. We found about 10 per cent. of typhoid bacilli alive after thirty minutes' exposure to this low temperature. Staphylococci were more resistant. Spores were scarcely killed at all.

Effect of High Temperatures.—Temperatures from 5° to 10° C. over the optimum affect bacteria injuriously in several respects. Varieties are produced of diminished activity of growth, the virulence and the property of causing fermentation are decreased, and the power of spore formation is gradually lost. These effects may predominate either in one or the other direction.

If the maximum temperature is exceeded the organism dies; the thermal death point for the psychrophilic species being about 37° C., for the mesophilic species about 45° to 55° C., and for the thermophilic species about 75° C. There are no non-spore bearing bacteria which when moist are able to withstand a temperature of 100° C. even for a few minutes. A long exposure to temperatures between 60° and 80° C. has the same result as a shorter one at the higher temperatures. Ten minutes' exposure to moist heat will at 60° C. kill the cholera spirillum, the streptococcus, the typhoid bacillus, and the gonococcus, and at 70° C. the staphylococcus, the latter being among the most resistant of the pathogenic organisms which have no spores.

Effect of Dry Heat.—When micro-organisms in a desiccated condition are exposed to the action of heated dry air the temperature required for their destruction is much above that required when they are in a moist condition or when they are exposed to the action of hot water or steam. A large number of pathogenic and non-pathogenic species are able to occasionally resist a temperature of over 100° C. dry heat for an hour. In any large number of bacteria a few are always more resistant than the majority. A temperature of 120° to 130° C. dry heat maintained for one and a half hours will destroy all bacteria, in the absence of spores.

Resistance of Spores to Heat.—Spores are far more resistant to all injurious influences than vegetative forms. They retain their power of germination for years without either nourishment or water, and are much more indifferent to the action of gases than bacilli, the spores of the anaërobic species being especially resistant to the action of oxygen. Spores possess a great power of resistance to both moist and dry heat. Dry heat is comparatively well borne, many spores resisting a temperature of over 130° C. for as long as three hours. Exposed to 150° C. for

one hour, practically all spores are killed. Moist heat at a temperature of 100° C., either boiling water or free-flowing steam, destroys the spores of known pathogenic bacteria within fifteen minutes; certain non-pathogenic species, however, resist this temperature for hours. The spores of a bacillus from the soil required five and a half to six hours' exposure to streaming steam for their destruction. They were destroyed, however, by exposure for twenty-five minutes in steam at 113° to 116° C. and in two minutes at 127° C.

The resistance of spores to moist heat is tested by suspending threads, upon which the spores have been dried, in boiling water or steam. The threads are removed from minute to minute and laid upon agar or in broth, which is then placed at a suitable temperature.

Practical Use of Heat Disinfection.—In the practical application of steam for disinfecting purposes it must be remembered that while moist steam under pressure is more effective than streaming steam it is scarcely necessary to give it the preference, in view of the fact that most known pathogenic bacteria produce no spores and the spores of the few that do develop them are quickly destroyed by the temperature of boiling water, and also that "superheated" steam is less effective than moist steam. When confined steam in pipes is "superheated" after its generation it has about the same germicidal power as hot, dry air at the same temperature. Esmarch found that anthrax spores were killed in streaming steam in four minutes, but were not killed in the same time by superheated steam at a temperature of 114° C. It should also be remembered that dry heat has but little penetrating power, and that even steam requires time to pass through heavy goods. Koch and Wolffhügel found that registering thermometers placed in the interior of folded blankets and packages of various kinds did not show a temperature capable of killing bacteria after three hours' exposure in a dry hot-air oven at 133° C. and over. We have often put a piece of ice in the middle of several mattresses and recovered it after exposing the goods to an atmosphere of live steam for ten minutes.

FRACTIONAL STERILIZATION.—Certain nutrient media, such as blood-serum and the transudates of the body cavities, as well as certain fluid foodstuffs, need at times to be sterilized, and yet cannot be subjected to temperatures high enough to kill spores without suffering injury. The property of spores, when placed under suitable conditions, to germinate into the non-spore bearing form, is here taken advantage of by heating the fluids up to 55° to 70° C. for one hour on each of six consecutive days, and keeping the fluid at about 20° C. during the intervals. By this means we kill, upon each exposure, all bacteria in the vegetative form, and allow, during the intervals, for the development of any still remaining in the spore stage, or which have reproduced spores, to change again into the vegetative form. Experience has shown that, with but few exceptions, an exposure for six consecutive days will completely sterilize the fluids so exposed.

With the usual culture media a temperature of 100° C. for twenty minutes does little or no harm while one of 115° C. is deleterious. With

heating to 100° C. an exposure on three consecutive days, and to 115° C. one or two suffices.

Pasteurization.—It is sometimes undesirable to expose food, such as milk, to such a temperature as will destroy spores, because of the deleterious effects upon food values of such high temperatures, and yet where a partial destruction is necessary. In these cases we heat the foodstuffs for from five to thirty minutes to such a temperature (70° C.) as will kill most of the bacteria in the vegetative form, but allow the spores to remain alive. Even this amount of sterilization kills about 90 to 98 per cent. of the bacteria. The exposure to this degree of heat alters the chemical composition of the milk but little.

CHAPTER V.

THE MATERIALS AND METHODS USED IN THE CULTIVATION OF BACTERIA.

The methods for the artificial cultivation of bacteria are of fundamental importance in bacteriology. The study of the characteristics of any bacterium requires that it be examined growing apart from all others. In order to separate one species from others and to study its morphological, biochemical, and cultural characteristics we have to prepare a number of sterile, solid, and liquid media and employ them in various technical ways. Before we can get a suitable growth of any special variety of bacteria, we must have the substances necessary for their growth present in the proper proportion and concentration. Different species of bacteria require very different foodstuffs, so that for each kind the proper food must be found through experimentation.

Preparation of Culture Media.—The most commonly used media have as their basis the watery extract of meat and peptone. The addition to this by Koch of gelatin gave us a transparent solid medium which had, however, the objection of melting below the temperature required for the growth of many pathogenic bacteria. Another substance of vegetable origin (agar) was found, which melted just below the boiling point of water. This has been substituted for gelatin whenever we desire to grow bacteria at temperatures above 20° C. or desire other characteristics of the agar media.

Preparation of Meat Infusion.—One pound (500 grams) of finely chopped, fresh, lean meat is macerated in 1000 c.c. of water and put in an ice-chest for from eighteen to twenty-four hours; or it may be warmed at a temperature not exceeding 60° C. for one hour. Any fat present is skimmed off. The last traces can be removed by stroking the surface with filter-paper. The infusion is now strained through a fine cheesecloth into a flask, and the remaining meat placed in a cloth and squeezed by hand or in a press. The resulting fluid contains the soluble albumin, the soluble salts, extractives, and coloring matter of the meat. This meat extract is then exposed to live steam, either without pressure in the Arnold steam sterilizer (Fig. 21) for thirty minutes, or, if the changes produced by a temperature of 110° to 115° C. are not objectionable, in the autoclave at one atmosphere of pressure for fifteen minutes, or boiled over a free flame for ten minutes. During this process all albumins are coagulated. While still hot the fluid is filtered through filter-paper or through absorbent cotton, and the reaction is tested and sufficient normal hydrochloric acid solution or sodium hydroxide added to give it the desired reaction, which is for most bacteria slightly alkaline to litmus. If in the boiling there has been any evaporation, sufficient

water is added to bring the fluid up to its original bulk. If the fluid is clear it is put into flasks and tubes and sterilized; if not clear, the white of one or two eggs is added to the fluid after cooling it down to about 55° C. After thoroughly mixing the eggs, the bouillon is boiled briskly for a few minutes, its reaction adjusted, and then again filtered and distributed in flasks and put in the Arnold sterilizer for one hour on each of three consecutive days, or in the autoclave for twenty minutes for sterilization. Instead of meat 2 to 4 grams of Liebig's or some other meat extract are added to each litre of water. For some purposes the extract is as good as the fresh meat, but for others it is inferior. This infusion contains very little albuminous matter, and consists chiefly of the soluble salts of the muscle, certain extractives, and any slight traces of soluble proteid not coagulated by heat. It is not, therefore, a suitable medium for most bacteria.

FIG. 21

Arnold steam sterilizer.

We use this as a basis for the following more useful media:

BOUILLON MEDIA.—These consist of meat infusion plus certain substances.

(*a*) *Peptone or Nutrient Bouillon.*—This has the composition: meat infusion, 1000 c.c.; sodium chloride, 5 grams; peptone (Witte), 10 grams. Warm moderately and stir until the ingredients are dissolved, then boil for thirty minutes in the Arnold sterilizer or the autoclave and treat as in making meat infusion. For the careful study of bacteria the exact reaction of the media should be carefully determined. For this purpose standard solutions are used with phenolphthalein or litmus as an indicator. This subject will be taken up in detail at the end of the chapter. For water bacteria sodium chloride is omitted and the reaction is made +1 per cent.

(*b*) *Sugar Bouillon.*—To the peptone broth after its completion 1 to 2 per cent. of glucose, lactose, saccharose, or other sugars is added. No more boiling than necessary to sterilize should be used after the addition of the sugars, since they become altered by heat. Temperatures higher than 100° C. should never be employed. These media are used to determine the effect of bacteria upon the different sugars.

(*c*) *Glycerin-peptone Bouillon.*—After filtration, 3 to 5 per cent. of glycerin is added to the peptone bouillon and the whole again sterilized. This medium is used especially for the growth of the tubercle bacilli.

(*d*) *Mannite-peptone Bouillon.*—This is prepared by adding 1 per cent. mannite to the peptone bouillon. It is used especially in differentiating

the varieties of dysentery bacilli, some fermenting mannite and others not. In careful work the bouillon must be rendered sugar free.

PEPTONE SOLUTION (Dunham's).—This is a simple 1 to 2 per cent. solution of peptone in tap or distilled water to which 5 per cent. of sodium chloride is added. The peptone and sodium chloride are dissolved by heating. The fluid is filtered, placed in tubes, and sterilized. The reaction is slightly alkaline to litmus and suitable for most purposes. It can be altered or standardized if desired.

(*a*) *Sugar-peptone Solution, etc.*—The various sugars, mannite, inulin, and glycerin are added to the peptone solution just as previously described for bouillon.

(*b*) *Sugar-free Bouillon.*—A quantity of a culture of bacillus coli or of bacillus lactis aërogenes is added to the meat extract and incubated at 37° for twenty-four hours. The acidity is neutralized, peptone and salt added, and treated as described under bouillon.

GELATIN MEDIA.—These are simply the foregoing bouillon and peptone media to which gelatin is added as follows: To the bouillon already prepared as described add 10 per cent. of sheet gelatin and neutralize. Add the whites of two eggs for each litre, and boil for a few minutes. Filter, place in tubes or flasks, and sterilize. Instead of adding gelatin to bouillon already prepared, it may be added to the meat infusion at the same time the peptone and salt were added in preparing nutrient bouillon as just described. Different preparations of gelatin differ greatly as to their melting point. Boiling lowers the melting point, so that heat should not be applied any longer than necessary. The melting point of different samples of nutrient gelatin varies between 20° to 27° C. The "gold-label" gelatin is employed.

AGAR MEDIA.—These are the various bouillon and peptone media to which 1 to 2 per cent. of agar are added. In order to lessen the effect of heat on sugars simple nutrient agar is first prepared and then the sugar added. They are prepared by adding to stock bouillon 1 or 2 per cent., as desired, of thread agar, melting it by placing over a free flame or in the autoclave or steam sterilizer. When the agar is brought into solution over a free flame there may be considerable loss of fluid by evaporation. This should be compensated for by adding additional water before boiling. Agar may be added directly to the meat infusion along with the peptone and salt. Indeed, this is an advantage, as agar-agar is very difficult to bring into solution, and is not injured in the least by prolonged boiling. The agar may be added to water alone in double the amount finally desired. To this is added an equal quantity of nutrient broth, which is also double its usual strength. Glycerin agar is simply nutrient agar plus 3 to 6 per cent. of glycerin. It is added to the hot nutrient agar just previous to putting it in the flasks. Nutrient agar begins to thicken at a fairly high temperature, and should be filtered as hot as possible. When small amounts are made it is well to place the filter and receiving flask in the sterilizer while filtering.

MILK.—This fluid is a good culture medium for most pathogenic bacteria. It should be obtained as fresh as possible, so that but little

bacterial change has occurred. It is first put in the ice-chest for twelve hours to allow the cream to rise. The milk is then siphoned off from below the cream into a flask and its reaction tested. After correction it is put in tubes or flasks and sterilized. If acid to phenolphthalein, normal sodium hydrate should be added to make it —1 per cent.

LITMUS MEDIA.—When it is desirable to determine whether bacteria produce in their growth acid or alkali from one or more of the constituents of the media litmus is frequently added. To prepare the litmus solution take the lump litmus, powder finely, and boil with distilled water so that a saturated solution is obtained. Filter and then boil for thirty minutes on two successive days. The litmus solution is added to the neutral media in sufficient quantity to give the desired depth of color. The less heating that is done after mixing the better the results. Merck's purified litmus in 1 per cent. aqueous solution should be used in careful work.

PETRUSKY'S LITMUS WHEY (as modified by Durham).—Fresh milk is slightly warmed and clotted by means of essence of rennet. The whey is strained off and the clot is hung up to drain in a piece of muslin. The whey, which is somewhat turbid, is then cautiously neutralized with 4 per cent. citric acid solution, neutral litmus being used as an indicator. When it gives a good neutral violet color with the litmus it is heated at 100° C. for one hour; thereby nearly the whole proteid is coagulated. It is thus filtered clear, and neutral litmus is added to a convenient color for observation.

NEUTRAL RED.—This dye is added to the peptone and bouillon-sugar media to the amount of 1 to 5 per cent. of a concentrated solution. Its reduction by the growth of bacteria is a valuable point in differentiation.

NITRATE BOUILLON.—Dissolve 10 grams of peptone in 1 litre of spring or tap water and add 0.02 grams of potassium nitrate (which is free of nitrites). This is placed in test-tubes and sterilized.

POTATOES.—Potatoes are used for some special purposes. The potatoes may, after thorough scrubbing and removal of "eyes," be soaked in bichloride of mercury (1:1000) for twenty minutes, and then sterilized on three consecutive days for one-half hour in the steam sterilizer. To use they are cut in thick slices and put in deep Petri dishes. For more careful work the potatoes are first cut into proper sizes for tubes, and then soaked for twelve hours in running water to remove the acidity.

BLOOD AND BLOOD-SERUM MEDIA. (*a*) *Fresh Blood Media.*—These are made by streaking sterile defibrinated or fresh human, rabbit, or other blood over nutrient agar contained in tubes or dishes. Sometimes fresh blood is added to fluid nutrient agar at 40° C. or to bouillon and a mixture thus obtained. Media made with fresh blood contains not only the hæmoglobin, but also intact red blood cells. Blood media are used for the growth of the influenza bacillus, for pneumococci and other bacteria, and for the observation of the reduction of the hæmoglobin by the growth of certain bacteria.

(*b*) *Heated Blood Media.*—The clot containing the red cells, after the separation of the serum, is broken up and added to bouillon and heated to 80° to 90° C. This makes a muddy fluid which is fitted only for the development of bacteria where no observation of their growth is required.

BLOOD-SERUM MEDIA. ASCITIC OR PLEURITIC FLUID.—Blood serum may be sterilized by fractional sterilization and remain fluid, or it may be rendered solid by the degree of heat used in sterilizing. The blood is obtained from an ox, horse, sheep, dog, or rabbit and collected into jars, flasks, or tubes. When it is to be used in a fluid state it should be drawn in an aseptic manner into a flask from a vein by means of a sterile cannula and rubber tube. When it is to be solidified, less care is necessary. It is here sufficient to catch the blood from the cut artery or vein into sterile jars or tubes. To facilitate clotting it is well to have in the jar or tube something for the clot to contract around.

Loeffler's Blood Serum.—Three parts of a calf's or sheep's blood is mixed with one part of neutral peptone bouillon containing 1 per cent. of glucose. The serum mixture is run into tubes, which are plugged and then placed in a slanting position in the serum inspissator.

Serum may be solidified and still remain translucent at a temperature of 76° C., but when heated to a higher degree a more definite coagulation takes place, and the medium becomes opaque. Care must be taken in coagulating blood serum at the higher temperature to run the temperature up slowly, and not to heat above 95° C. until the serum has firmly coagulated; for, unless these precautions are taken, ebullition is likely to occur, which will lead to the formation of bubbles and an unevenness of the surface upon which growth is to be obtained and studied. Serum may be solidified at the temperature mentioned in an incubator, water-oven, or even in an Arnold sterilizer with the top covered by a cloth instead of the usual lid, and when coagulated firmly (90° C.) the tubes and their contents may, on the following day, be sterilized in streaming steam at 100° C. without danger of the subsequent formation of bubbles. Koch's serum coagulator (Fig. 22) is, however, the most convenient apparatus. Some bacteriologists prepare the tubes of solidified serum in the autoclave, gradually increasing the temperature to 110° C. This is a very rapid and convenient method. It has seemed to us, however, that the high temperature injured the medium somewhat.

FIG. 22

Blood-serum coagulator.

Alkaline Blood Serum.—To each 100 c.c. of blood serum add 1 to 1.5 c.c. of a 10 per cent. solution of sodium hydrate. Treat as Loeffler's serum. This will give a solid, clear medium consisting chiefly of alkali albumin.

Serum-bouillon Media (Marmorek's Media):

1. Human serum, 2 parts; nutrient bouillon, 1 part.
2. Ascitic or pleuritic fluid, 1 part; nutrient bouillon, 2 parts.
3. Horse serum, 1 to 2 parts; nutrient bouillon, 1 to 2 parts.

These media were first used extensively by Marmorek in cultivating streptococci. The ascitic fluid bouillon has been found by Williams to be of great use in enriching cultures of diphtheria bacilli. It is also the best medium for the growth of pneumococci, streptococci, and many other pathogenic bacteria.

Serum-water Media (Hiss' Serum Media).—When diluted with 2 to 10 parts of water, many sera can be steamed without coagulating.

1. Ox serum, 1 part; distilled water, 2 parts; normal sodium hydrate, 0.1 per cent.
2. The same, with inulin 1 per cent. substituted for the sodium hydrate.

For the sterilization of undiluted fluid serum and of ascitic and pleuritic fluids, it is requisite that it be exposed to a temperature of from 62° to 66° C. for one hour on each of six consecutive days. The best apparatus for obtaining and maintaining this temperature (about 65° C.) is a small and well-regulated incubator or chamber surrounded by a water space, into which the tubes and flasks containing serum are to be put each day, and in which they are to be left for the prescribed time after having been warmed to the desired temperature.

Serum may be preserved by placing it in flasks which, after the addition of 5 per cent. of chloroform, are sealed. When it is to be used it is filled into sterilized culture (test) tubes and sterilized by exactly the same methods as are employed in sterilizing fresh serum. The chloroform, being volatile, tends to disappear at ordinary temperatures, but is quickly and surely driven off at the temperatures used in sterilizing.

Serum may be efficiently sterilized, when great care is used, by passing it through a well-tested Pasteur filter, under pressure. When so treated the fluid is very clear and light-colored.

Important media used for special varieties of bacteria will be noted in the chapters devoted to them.

Reaction of Culture Media.—The reaction of media is a matter of the greatest importance, since slight variations will often aid or inhibit the growth of bacteria and also produce marked differences in the microscopic and macroscopic characters of a growth. Hence it is important to work with media whose reaction is accurately known, so that necessary variations or uniformity can afterward be attained

Formerly it was customary to use litmus as the indicator in neutralizing media, adding normal soda or hydrochloric acid solution until the red litmus turned slightly blue, or the blue litmus just a tinge less blue. This was considered the neutral point. This is still a satisfactory method for those who are only going to cultivate the common pathogenic bacteria for diagnostic purposes or for the development of toxin. Most parasitic bacteria which grow at all on artificial culture media develop best in them when they have a neutral or slightly alkaline reaction to litmus. If a certain alkalinity is desired a definite number of cubic centimetres

of normal soda solution can be added for each litre of neutral media; if an acidity is desired, normal hydrochloric acid solution is added.

Many bacteriologists consider that litmus is not delicate enough to be entirely satisfactory, especially when experiments are to be reported or exactly repeated. This objection is made chiefly by those investigating water bacteria who are watching cultural and biochemical characteristics in simple peptone-beef media. For these purposes phenolphthalein has been generally selected. It is of great importance to remember that different indicators not only differ in delicacy, but that they react differently to different substances. A medium which is alkaline to litmus is acid to phenolphthalein, showing that there are present in such media substances possessing an acid character which litmus does not detect. These substances are weak organic acids and organic compounds, theoretically amphoteric, but in which an acid character predominates. Thus, a litre of bouillon becomes, on the addition of 1 per cent. of peptone, more alkaline to litmus, but decidedly more acid to phenolphthalein; 100 c.c. of water with 1 per cent. of peptone is acid to phenolphthalein to such an extent that 3.5 c.c. of decinormal NaOH is required to neutralize it. To litmus it is alkaline and requires 3.4 c.c. of decinormal HCl. Two per cent. of peptone doubles the difference. The same figures hold approximately true for peptone broth. We should find by growing the bacteria just what reaction we want for any variety, and then test the fluid with phenolphthalein or litmus as the indicator. With precisely similar ingredients we can then exactly reproduce at any time in the future the same reaction, but with different materials one would again have to study the reaction.

Titration of Culture Media.—We must have accurately standardized normal and decinormal solutions of sodium hydrate and hydrochloric acid; also a 0.5 per cent. solution of phenolphthalein in 30 per cent. alcohol and a neutral 1 per cent. solution of Merck's litmus.

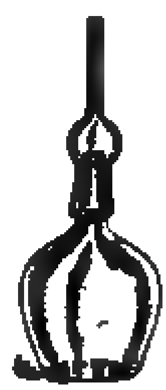
Care should be taken to prevent the absorption of carbon dioxide by the soda solution, by arranging that all air which comes in contact with the latter, either in the stock bottle or in the burette, shall first pass through a strong solution of sodium or barium hydrate. The arrangement of the apparatus is described in any work on chemical analysis. The medium is brought to the desired volume with water, and boiled four minutes to expel the carbon dioxide. Media are commonly warm or hot when measured, hence it must be remembered that true volumes cannot be thus obtained; for instance, a litre measured at, say, 80° C. would be only 973 c.c. if measured at 20° C., the temperature at which litre flasks are calibrated. Since many media cannot be cooled to 20° C. because of solidification, as in the case of agar or gelatin, it is a better plan when accuracy is important to determine measures of volume by weight. For this, place a clean, dry saucepan, in which the medium is to be prepared, upon one side of a trip scale, and counterbalance its weight exactly. The weight of a litre of bouillon, gelatin, or agar having been determined once for all, the necessary weights added to the weight of the pan will give the amount which the pan and its con-

tents must balance when the volume is exactly one litre. A portion of the medium brought to the exact volume is then taken and cooled to room temperature (20° C.), or to a point a few degrees above solidification, and 10 c.c. withdrawn, placed in a small beaker, 50 c.c. of distilled water and 1 c.c. of the phenolphthalein solution added. If the medium is acid the $\frac{N}{10}$ NaOH solution is then run in cautiously until a pale but decided pink color is obtained. The number of cubic centimetres of the solution used, multiplied by ten, will give the number of cubic centimetres of normal sodium hydrate per litre necessary to effect complete neutralization. The question as to what is the best reaction of media for general work is not an easy one to settle, and one on which bacteriologists differ. What is the proper reaction for one variety of bacteria is often far from the best for some other variety. Reactions are now commonly expressed by plus or minus signs, the former representing an acid and the latter an alkaline condition, the number following the sign representing the percentage of normal acid or alkali present in the medium. Thus, +1.5 would indicate that the medium contained 1.5 parts per 100 or 1.5 per cent. of free normal acid,

Fig. 23

Erlenmeyer flask.

Fig. 24



Pasteur flask.

while —1.5 would indicate that the medium contained an equivalent quantity of free alkali. The committee of the American Public Health Association in 1898 adopted a medium whose litre was +1.5 as the best for general work in water examinations. In 1905 this was changed to +1.0 per cent. A medium whose reaction is +0.5 per cent. acid to phenolphthalein is still better adapted for many bacteria. It cannot be too strongly impressed upon the reader that whatever the reaction, its measure should be stated in all descriptions of cultural characters. The litmus solution is added in the same way as that of phenolphthalein.

Storage of Media.—The nutrient media are stored in glass flasks (Fig. 23). From these, as needed, glass tubes are filled. When small amounts of media are taken frequently from flasks, Pasteur's flasks (Fig. 24) are of great convenience. They consist of a flask with a ground-glass neck, over which fits a cap. This cap may or may not terminate, as desired, in a narrow tube, which is plugged with cotton. The cap keeps the edges of the flask free from bacteria and prevents the cotton from

sticking. A tumbler or a simple cap of paper over the neck answers much the same purpose.

Preparation and Filling of Tubes.—The cheaper grades of test-tubes should be avoided. They are thin and break easily, and also frequently frost on heating from the separation of silicic acid. The tubes of the better class can be used after rinsing with hot water; cheap tubes are very alkaline and must first be soaked in dilute hydrochloric acid.

CLEANSING AND STERILIZATION OF APPARATUS.—In order to study bacteria, both in culture media and in the living body, we must, as already stated, separate those developed from one organism from all others and study them by themselves in pure cultures. In order to do this we have to take the greatest precautions to ensure that the materials that we make use of for the growth of bacteria, the flasks and tubes that hold these materials, and the instruments with which we transfer the bacteria are sterile. In bacteriological work sterilization is practically always done by means of dry and moist heat, for no antiseptic substances can be allowed to remain in any of the media used for the growth of bacteria or on any of the apparatus which would come in contact with them, as such substances would inhibit the growth of the bacteria which we desired to study.

The platinum wires and loops used in transferring bacteria are sterilized by holding them for a moment until red-hot in a gas or alcohol flame. They should not be used until time enough has elapsed for them to cool sufficiently not to injure the bacteria touched by them. Knives, instruments, etc., are, after thorough cleansing, placed in boiling 1 per cent. soda solution for three to five minutes. Hypodermic needles are sterilized by boiling in soda solution, or, when this is impossible, they are first frequently rinsed with boiling or with very hot water, and then filled with a 5 per cent. carbolic acid solution for at least thirty minutes and then rinsed again with sterile water. New tubes and flasks sometimes require to be washed in a 2 per cent. solution of nitric acid, so as to remove any free alkali which may be present. They are finally thoroughly rinsed in pure water. Old tubes, flasks, and other glassware are boiled for about thirty minutes in a 5 per cent. solution of washing soda, and then thoroughly rinsed off with water until perfectly clean. If necessary, any dirt clinging to the insides of the flasks and tubes can be removed by bristle brushes or suitable swabs. After the tubes and flasks have been thoroughly cleaned they are plugged loosely with ordinary cotton-batting, or, if that is not at hand, the more expensive absorbent cotton. The tubes and flasks with their cotton plugs and all other glassware are sterilized by dry heat at 150° C. for one hour in the dry-heat sterilizer (Fig. 25).

FIG. 25

Dry-heat sterilizer.

The sterile tubes and flasks are filled with the media, when small

quantities are used, by means of a glass funnel. The main precaution to be observed is not to let the media soil the neck of the tubes and flasks, as this would cause the fibres of the cotton plugs to adhere to the sides of the tubes when the media dried, and make it difficult to remove the plugs wholly when we wished to inoculate the contents of the tubes.

The tubes and flasks, plugged with sterile cotton and full of media, are put in the steam sterilizer for one-half hour on three consecutive days, or in the autoclave for fifteen minutes for two consecutive days. A portion of the tubes containing nutrient agar are laid in a slanted position before cooling, after the final sterilization, so that a larger surface may be obtained.

THE CULTIVATION OF BACTERIA.

Bacteria can seldom be identified by their microscopic and staining characteristics alone. We can only study their forms, arrangement, and motility or lack of motility. To go beyond this we have to grow the micro-organism in pure culture on the various culture media and perhaps also in animals. It is necessary, also, to have the proper conditions as to temperature, moisture, access of oxygen, etc.

When we make cultures from any material, we are very apt to find that instead of one variety of bacteria only there are a number present. If such material is placed in fluid media contained in test-tubes, we find that the different varieties all grow together and become hopelessly mixed. When, on the other hand, the bacteria are placed on solid media they develop about the spot where they were inoculated. If different varieties, however, are placed too near together, they overgrow one another; it is thus advisable to have a greater surface of nutrient material than is given on the slanted surface of nutrient agar or blood-serum contained in test-tubes. This need is met by pouring the media while warm on flat, cool, glass plates or into shallow dishes.

Technique of Making Plate Cultures.—In making plate cultures two methods are carried out. In the first the material with its contained bacteria is scattered throughout the fluid before it hardens; in the second it is streaked over the surface of the medium after it has solidified. Nutrient agar and nutrient gelatin, the two substances used for plate cultures, differ in two essential points, which cause some difference in their uses. Nutrient 1 per cent. agar melts near the boiling point and begins to thicken at about 36° C. It is not liquefied by bacterial ferments. Nutrient 10 per cent. gelatin melts according to the variety used, at the low temperature of about 23° to 27° C., and solidifies at a point slightly below that. It is liquefied by many bacterial ferments. When we wish to inoculate fluid nutrient agar for plate cultures we have to take great care that in cooling it to a point which will not injure the bacteria, about 41° C., we do not allow it to cool too much and thus solidify and prevent our pouring it into the plates. To prevent this, when a number of tubes are to be inoculated, they are placed while still

hot in a basin of water which has been heated to about 45° C. When the temperature of the agar in the tubes, as shown by a thermometer in one, has fallen to 42° C., the water, milk, feces, bacterial culture, or other substance to be tested is added to the other tubes in whatever quantity is thought to be proper up to 1 c.c. A greater quantity of fluid would dilute and cool the nutrient agar too much. After inoculation the contents of the tubes are thoroughly shaken and poured out quickly into round, flat-bottomed, glass Petri dishes (Fig. 26), the covers of which are removed for the required time only. Instead of placing the substance in the tube it is often placed directly in the Petri dish. The melted nutrient gelatin or agar is thus poured over it and by gently tipping the dish mixed with it. The bacteria are now scattered throughout the fluid, and as it quickly solidifies they are fixed

FIG. 26

Petri dish.

FIG. 27

Photograph of a large number of colonies developing in a layer of gelatin contained in a Petri dish. Some colonies are only pinpoint in size; some as large as a pencil. The colonies here appear in their actual size.

wherever they happen to be, and thus as each individual multiplies clusters are formed about it at the spot where it was fixed at the moment of solidification. The number of colonies of bacteria (Fig. 27) thus indicate to us roughly the number of living bacteria in the quantity of fluid added to the liquid agar. Groups or chains of bacteria which in spite of shaking remain attached produce single colonies. Nutrient gelatin is used exactly as agar, except that as the average product does not congeal until cooled below 22° C. we have no fear of its cooling too rapidly. In order not only to count the number of colonies which develop, but also to obtain a characteristic growth, it is desirable not to have them too near together.

Dilution Methods.—As it is impossible to know the number of bacteria in any suspected fluid, it is usual to make a set of four different plates, to each of which a different amount of material is added, so that some one of the four will have the required number of colonies. The dilutions are made in sterile distilled water or bouillon. In the first tube we place an amount which we believe will surely contain sufficient and probably too many bacteria. To the second tube we add 10 per cent. of the amount added to the first, and to the third 10 per cent. of the second, and to the fourth 10 per cent. of the third. Thus, if the first contained 60,000 colonies the second would have 6000 (Fig. 27), the third 600, and the fourth 60. If, on the other hand, the first contained but 60, the second would have about 6, and the remaining two would probably contain none at all. When there are many colonies present the dishes are covered by a glass plate (Fig. 29), ruled in larger and smaller squares, Wolffhügel's apparatus. With the eye or aided by a hand lens the colonies in a certain number of squares are counted and then the number for the whole contents estimated.

FIG. 28

Well-distributed colonies on agar in Petri dish.

FIG. 29

Wolffhügel's apparatus for counting colonies.

When the material to be tested is crowded with bacteria it is often best to make an emulsion of a portion of it, and use this rather than the original substance for making the dilutions to be used. Measured quantities of the diluted material can be transferred most accurately through a sterilized, long, glass pipette graduated in one one-hundredth cubic centimetres, or, more roughly, by a platinum loop of known size.

The nutrient agar-agar is frequently used in a different manner. About 8 c.c. are poured into the Petri dish and allowed to harden. The substance to be tested bacteriologically, or a dilution of it, is then drawn across the medium in a series of parallel streaks by means of a platinum loop lightly over its surface. Each successive streak is made with the same needle or loop without replenishing the material to be tested. Each streak will therefore leave less deposit of bacteria and fewer colonies will develop. While in the former method most of the bacteria developed under the surface, here all develop upon it. This

is an advantage, as many forms of bacteria develop more characteristically on the surface than in the midst of the media, and it is easier to remove them free from other bacteria with the platinum needle. Instead of streaking the material by means of the platinum wire over the agar, a loopful may be deposited on the agar and then smeared over its surface by a sterile swab or bent glass rod. The method of using glass plates upon a cooling stage has now been practically given up for the more convenient one of Petri dishes. In warm weather the dishes may be cooled before using, so as to harden quickly the agar or gelatin that is poured into them.

An old method, which is still sometimes used to find the number of living bacteria, is, instead of pouring out the media which has been inoculated, to congeal it on the sides of the test-tubes. This is best done by laying the tube flat on its side on a cake of ice and rotating it. Tubes come especially formed for this by having a slight neck, which prevents the media running up to the plugged end of the tube. This method, Esmarch's, is used only when the Petri dishes are not obtainable or cannot easily be transported.

Study of Colonies in Plate Cultures in Nutrient Agar.—The plates should be removed after twelve to twenty-four hours' growth at blood temperature and after one to three days at 70° F. (21° C.). The special time allowed varies according to the rapidity of the growth of the varieties developing; thus, bacteria, such as the streptococci and influenza bacilli, reach the characteristic development of their colonies in from ten to sixteen hours, while others continue to spread for several days. If we wait too long where numerous varieties of bacteria are growing the colonies of heavier growth may cover up the finer and more delicate ones. As a rule, the younger colonies are more characteristic, except where the development of pigment is sought.

The colonies are first examined with the eye (Fig. 27), then with magnification of about 60 diameters (Figs. 30 to 35), and then again, if necessary, at from 400 to 500 diameters (Fig. 44). We note everything we can about them, such as their size, surface elevation, form, internal structure, edges, and optical characters. If grown in gelatin, whether they have or have not caused liquefaction. The accompanying schematic representations from Lehman and Neumann (Figs. 36 to 43) illustrate some of these points.

At the higher magnification we begin to detect the individual bacteria (Fig. 44). After studying the colonies we remove a few of the bacteria from one or more of them by touching them with the tip of a sterile platinum needle (Fig. 45), and thus transfer them to a cover-glass for microscopic examination, or to new media where they may develop in pure cultures and show their growth characteristics.

Hanging Block Cultures.—In order to study the morphology and manner of multiplication of bacteria to better advantage than in the hanging drop, Hill devised the following procedure: Melted nutrient agar is poured into a Petri dish to a depth of about one-eighth to one-quarter of an inch. When cool a block is cut out about one-

quarter of an inch square. The block is placed under surface down on a slide and protected from dust. A suspension of the growth to be examined is then made in sterile bouillon and spread over the upper

FIG. 30

FIG. 31

FIG. 30.—Irregular fringed colony (B. malignant œdema). (From Kolle and Wassermann.)
FIG. 31.—Round surface colony (colon bacilli grown in stiff gelatin).

FIG. 32

FIG. 33

FIG. 32.—Colony of typhoid in rather stiff gelatin.
FIG. 33.—Colonies of typhoid and colon bacilli in rather soft gelatin.

FIG. 34

FIG. 35

FIG. 34.—Colony of colon bacilli grown in soft gelatin.
FIG. 35.—One irregular colony of colon and two of typhoid bacilli in soft gelatin. (Figs. 31–35 from photographs by Dunham.)

surface of the block. The slide and block are then put in the incubator for ten minutes to dry slightly. A clean cover-slip is now placed on the agar block in such a way as to avoid large air bubbles. The slide

is then removed. With the aid of a platinum loop a drop or two of melted agar is run along each side of the block to fill any angles between

FIG. 36 FIG. 37

FIG. 36.—Moist raised colonies with no visible structure, looking like a drop of water.

FIG. 37.—Deep colonies, usually either light brown, gray or yellow in color, opaque, with little marking. (Figs. 36–43 from Lehman and Neumann.)

FIG. 38 FIG. 39 FIG. 40

FIG. 38.—The colonies very finely granular, with or without twisted threads at borders.

FIG. 39.—Colonies opaque in centre with lighter borders. The margin is coarsely granular.

FIG. 40.—Colony in gelatin. The centre is coarsely granular in partly fluid gelatin. The borders are formed of wavy bands of threads.

FIG. 41 FIG. 42 FIG. 43

FIG. 41.—Colonies circular in form, composed of radiating threads.

FIG. 42.—Colonies with opaque centres, with a thin border fringe.

FIG. 43.—Colony showing a network of threads which is thicker in centre.

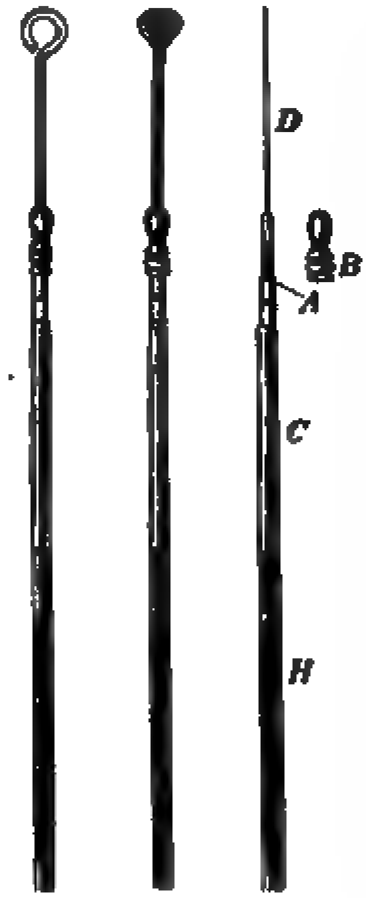
it and the cover-glass. After drying in the incubator for five minutes it is placed over a hollow slide and sealed with paraffin.

In using nutrient gelatin one must always remember not to allow it to stay where the temperature is over 20° C., for if that happens the

FIG. 44

Two surface colonies of diphtheria bacilli upon agar. × 500 diameters.

FIG. 45



Platinum needle, loop, and spade.

FIG. 46

Stab cultures of three cholera spirilla in gelatin, showing in upper portion of growth considerable liquefaction of nutrient gelatin.

media as a rule will melt; nor must the liquefying colonies be allowed to grow for too long a time, or the entire media will become fluid.

Pure Cultures. — If we transfer without contamination bacteria from a colony formed from a single organism to new media, and these grow, we have what we call a pure culture of that variety. When these are transferred to the solid media we call the growth which takes place from smearing the bacteria over the surface a surface or smear culture, and that formed in the depth of the media by plunging the needle carrying the bacteria into it a stab culture (Figs. 46 and 47).

In transferring bacteria from one tube to another we slant the tubes so that no dust may fall within and contaminate with other bacteria the special variety we wish to transplant. The greatest care must be taken that the sterilized platinum needle used to transfer the bacteria is not infected by touching any non-sterile matter. The upper rim of culture tubes should be passed through the flame so as to destroy any bacteria resting there. Even with our utmost care bacteria will from time to time pass from the air or edges of our tubes into the culture

media, and thus possibility of contamination must always be kept in mind. When it occurs upon solid media we, as a rule, easily detect it, for we notice the growth at some point of bacteria of different colony characteristics; but in fluid media, on account of the complete mingling of the bacteria, we are not so apt to notice the additional growth.

THE STUDY OF PURE CULTURES IN TUBED MEDIA.—A few points of the many which should be observed are the following:

Gelatin stab cultures.

A. Non-liquefying.

Line of puncture.

Filiform, uniform growth, without special characters.

Beaded, consisting of loosely placed, disjointed colonies.

Arborescent, branched, or tree-like.

Some of these points are illustrated in Fig. 47, sketched by Chester

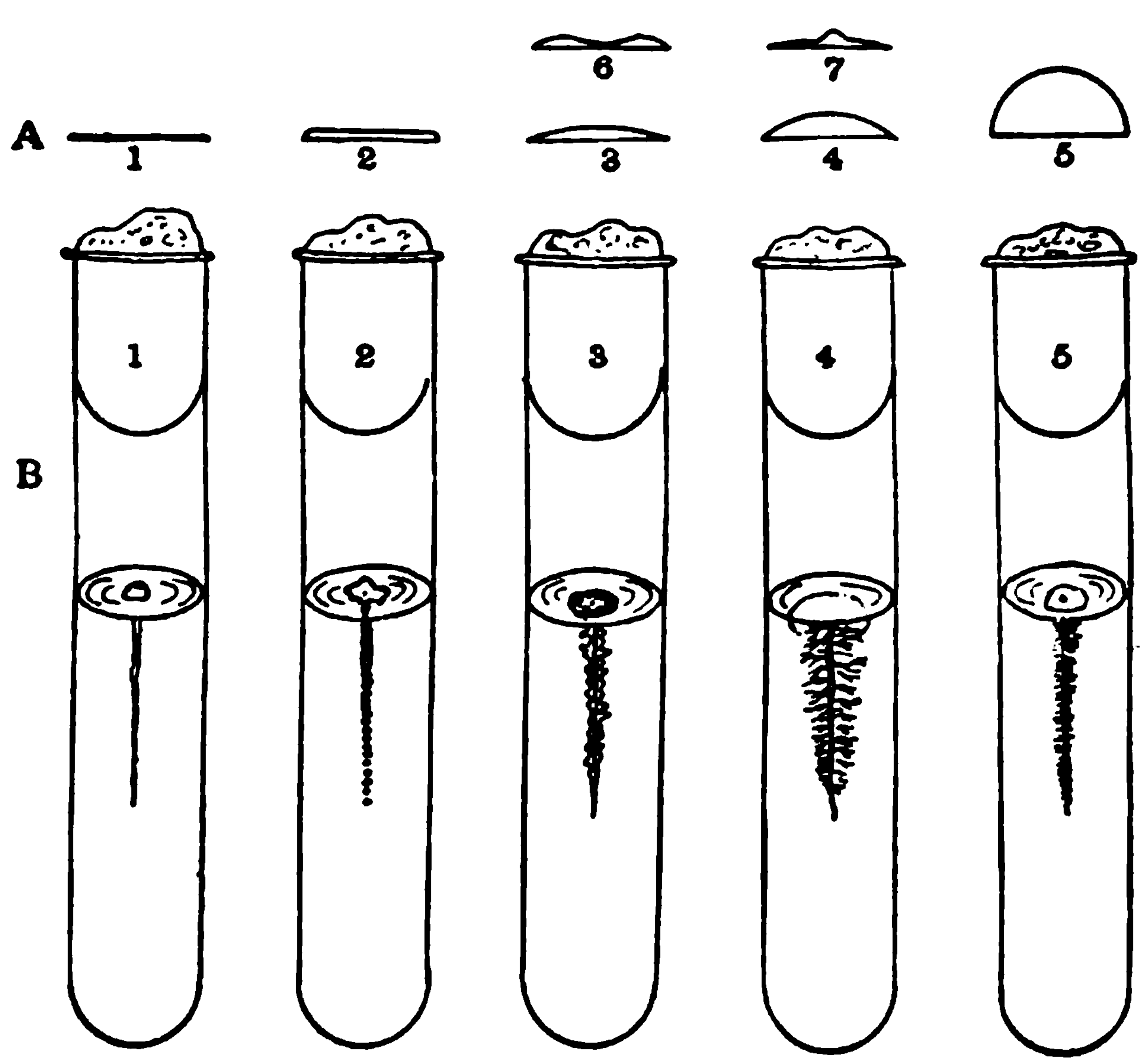
B. Liquefying.

Crateriform, a saucer-shaped liquefaction of the gelatin.

Saccate, shape of an elongated sac, tubular (Fig. 46).

Statiform, liquefaction extending to the walls of the tube.

FIG. 47



Showing characters of gelatin stab cultures: *A*. Characters of surface elevation: 1, flat; 2, raised; 3, convex; 4, pulvinate; 5, capitate; 6, umbilicate; 7, umbonate. *B*. Characters of growth in depth; 1, filiform; 2, beaded; 3, tuberculate-ecinulate; 4, arborescent; 5, villous. (From Chester.)

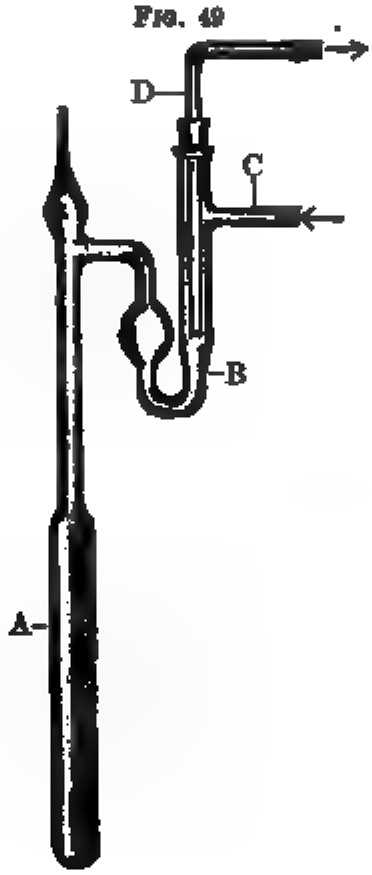
Nutrient agar tube cultures give fewer points for observation, but should be studied in the same way. The agar in the tubes is usually slanted and the culture growth is not only in the stab, but along the streaked surface. The characteristics of each should be noticed.

Apparatus for Obtaining a Suitable Temperature for the Growth of Bacteria. INCUBATORS.—In order to have a constant and proper temperature for the growth of bacteria, forms of apparatus called incubators have been devised (Fig. 48). These consist, in their simplest form, of an inner air chamber surrounded by a double copper wall containing water. The apparatus externally is lined with asbestos, to prevent radiation. It is supplied with doors and with openings for thermometers and a thermoregulator. The thermoregulators are of various kinds; those in most use depend upon the expansion or contraction of the fluid in the bulb *A* (Fig. 49), which rests within the water-jacket, to lessen or increase the space between the surface of the mercury *B* and the inner tube *D*, thus allowing of the passage of a greater or less

FIG. 48

Small incubator.

FIG. 49



Thermoregulator.

quantitity of gas to the burner through the tube *D*. Other forms depend upon the contraction or expansion of metal, or the use of the electric current to control the flow of the gas.

The temperature in the air chamber is kept above that of the surrounding air by means of a gas flame regulated as above described, or, when that cannot be obtained, a lamp.

The temperature is reduced by passing a stream of cool water through the water chamber, which is itself regulated. When very accurate investigations are to be made a gas-pressure regulator is added to the thermoregulator. Incubators are also both warmed and regulated by electricity.

In emergencies a culture may be developed at the blood temperature by placing it in water contained in a small vessel, which itself is contained in a larger vessel, also filled. By adding a little hot water

from time to time to the outer vessel the temperature can readily be kept between 34° and 38° C., which is sufficiently uniform for bacteria such as the diphtheria bacilli to grow.

As a temporary expedient during the night, when haste is necessary, it is possible, when the culture medium is solid and within a strong glass tube or metal case, to make use of the body heat by putting it under the clothing next to the body or sleeping upon it. Naturally, this should only be done when other means fail. Several times, when in the country, this method has enabled the writer to obtain a growth of diphtheria bacilli over night, and thus get important information, when otherwise it would have been impossible.

Methods for Obtaining Anaerobic Conditions for Bacteria.—Pasteur excluded the oxygen by pouring a layer of oil on the culture fluid. A simple device is that of Koch, who placed a thin strip of sterile mica upon the still fluid agar or gelatin in the Petri dish, which had already been inoculated. After the solidification of the media the portion under the mica is excluded from the air and anaërobic growth can develop.

A second simple method (Liborius) is to fill the tubes with media fuller than usual and to inoculate the bacteria deep down to near the bottom of the tubes while the media are still semisolid. An anaërobic growth will take place in the lower part of the tube. In a similar way the closed arm of the fermentation tube will suffice for anaërobic growth, if the opening connecting it with the open bulb is quite small. Wright devised the following procedure: A short glass tube with constricted ends is used. Each end has a piece of rubber tubing attached. One of these is connected with a glass tube, which projects through the cotton plug of the test-tube. The test-tube contains bouillon. The whole is sterilized and then the test-tube inoculated. The bouillon is then drawn up into the constricted tube, which is sealed by simply pushing down the tube so that both rubber ends are sealed by being bent on themselves. When spores are present, a simple method suggested, I believe, by McFarland, can be successfully employed. Vessels plugged with stoppers perforated by glass tubes drawn to a point are filled to such a height that when the fluid is heated to 80° C. it will just fill them. They are inoculated when the bouillon is at about 60° C., heated to 80° C., and then sealed by closing the tube's point by means of a flame. After inoculating and heating, instead of sealing the glass tube a sterile rubber cork can be inserted.

If much fermentation is expected, the cork should be clamped or tied to the bottle, so that it will not blow out. One advantage of this method is that any contaminating organisms which have no spores will be killed.

When sealed the bottles should be cooled and then placed in the incubator.

A very convenient modification of Pasteur's method for the growth of bacteria in fluid media is to cover the fluid with albolene or paraffin.

In boiling all the oxygen is driven out. We prepare all our tetanus toxins in this way: Litre flasks are filled to near the neck with bouillon. This is covered with a one-half inch layer of albolene or paraffin. The bouillon after boiling is quickly cooled by setting the flask containing it in a shallow layer of cool water, so as to lower the temperature of the lower portion of the bouillon to 40° C. or under, while leaving the paraffin on the surface still fluid. While in this condition it is inoculated with the tetanus culture. Bits of tissue suspected to contain tetanus bacilli are dropped into smaller flasks filled and prepared in the same way.

FIG. 50

Jar for anaërobic cultures.

FIG. 51

Buchner's anaërobic tube. The fluid consists of pyrogallic acid dissolved in 10 per cent. soda solution. By Wilson's method the tubes are charged with pieces of caustic potash covered with pyrogallic acid.

Displacement of Air.—In the more complicated methods the plates or tubes are placed in jars of a type devised by Novy (Fig. 50), in which the oxygen is displaced by a stream of hydrogen developed by the Kipp apparatus, through the action of pure granulated zinc and a 25 per cent. solution of pure sulphuric acid. When all the oxygen has been displaced the jars are sealed by rotating the stopper.

Absorption of Oxygen.—In another method the oxygen is extracted by a mixture of pyrogallic acid and caustic potash. To each 100 c.c. of air space in the jar 1 gram of pyrogallic acid and 10 c.c. of 6 per cent. solution of potassium hydroxide are added and the jars immediately sealed. A very simple procedure has been described by Wilson. In a large test-tube a small piece of solid caustic potash is placed and over this powdered pyrogallic acid is poured. A smaller culture tube with the desired medium is inoculated. Water is now added to the large test-tube, which works its way slowly through the pyro-

gallic acid. The small tube is quickly inserted and the whole sealed by water or a rubber cork (Fig. 51). Solid culture media in test-tubes can be inverted over the acid soda mixture, which is then covered by a layer of albolene to prevent the absorption of oxygen from the air. The displacement method is often used along with that of absorption.

ASSOCIATED WITH AËROBIC BACTERIA.—Anaërobic bacteria mixed with aërobic bacteria will grow in the apparent presence of oxygen, the aërobic bacteria robbing the media of it. Thus, tetanus and diphtheria grow together in an open flask of bouillon.

METHOD FOR ADAPTING BACTERIA TO ANIMAL FLUIDS.—The placing of cultures in collodion sacs in the abdomens of animals has been used extensively by the Pasteur school for exalting the virulence of bacteria or trying to adapt them to species of animals differing from the one from which they were isolated.

The underlying idea is to grow the organisms in the peritoneal cavity of an animal under such conditions that the waste products of the germs will be removed, an abundant supply of nutrient material furnished, and the germs themselves protected from the action of the phagocytes. The hermetically sealed collodion sacs answer this purpose. The collodion used is the U. S. Pharmacopœia solution, which by exposure to the air has been concentrated one-third.

The sealed inoculated sacs are to be inserted into the peritoneal cavity with every possible precaution for asepsis. The sacs are left in place for days or months, as the experiment requires.

CHAPTER VI.

MICROSCOPIC METHODS.

THE PREPARATION, STAINING, AND MICROSCOPIC EXAMINATION OF BACTERIA.

THE direct microscopic examination of suspected substances for bacteria can be made either with or without staining. Unstained, the bacteria are examined in a hanging drop or on transparent media, under daylight, or, better, artificial light to note their motility, their size and form, and their general arrangement; but for more exact study of their appearance they can be so much better observed when stained that this step is always advisable.

Elimination of Foreign Bacteria from Preparations.—Since bacteria are present in the air, in dust, in tap water, on our bodies, clothes, and all surrounding objects, it follows that when we begin to examine substances for bacteria the first requisite is that the materials we use, such as staining fluid, cover-glasses, etc., should be practically free from bacteria, both living and dead, otherwise we may not be able to tell whether those we detect belonged originally in the substances examined or only in the materials we have used in the investigation.

Film Preparation.—A cover-glass or slide preparation is made as follows: A very small amount of the blood, pus, discharges from mucous membranes, cultures from fluid media, or other material to be examined is removed by means of a sterile swab or platinum loop and smeared undiluted in an even, thin film over a perfectly clean,[1] thin cover-glass or slide. From cultures on solid media, however, on account of the abundance of bacteria in the material, a little of the growth is diluted by adding it to a tiny drop of clean distilled water which has been previously placed on the glass. The amount of dilution is learned after a few trials. It is best to add to the drop just enough of the culture to make a perceptible cloudiness. The mixture is then smeared thinly and uniformly over the glass. When blood or pus is to be studied it is well to put a small drop on a slide or cover-glass and then immediately to place on top of this another. The fluid will spread between the two, and when they are drawn apart a fairly even

[1] To render new cover-slips clean and free from grease, place them in strong nitric acid for a few hours, then rinse them in water, then in alcohol, then in ether. Place them finally for keeping in alcohol, to which a little ammonia has been added. When used wipe with soft, clean linen or cotton cloth. If old cover-slips are used, boil first in soda solution. Another procedure is to soak the cover-glasses first in alcohol, then wipe with soft linen, then place in a Petri dish, and heat in the dry sterilizer for one hour at 200° C. A cover-glass is not clean when a drop of water spread over it does not remain even, but gathers in droplets.

smear will be left on them. If it is desired to preserve the blood cells intact the films are placed in a saturated solution of corrosive sublimate for two to three minutes, and then washed in running water, or instead of sublimate exposed to the vapor of formalin.

When milk films are made they are, after fixation, cleared of fat by means of ether. From whatever source derived the film is allowed to dry thoroughly at the usual air temperature, and then, in order to fix the film with its contained bacteria to the glass, the latter is grasped in any one of the several kinds of forceps commonly used, and is passed three times by a rather slow movement through the Bunsen or alcohol flame. Instead of this method the film may be fixed to the glass by placing it in absolute alcohol for a few minutes. The smear thus prepared is usually stained either by the simple addition of a solution of an aniline dye, for from one to five minutes, or by one of the more complicated special stains described later. When the stain is to be hastened or made more intense the dye is used warm. For ordinary staining the bacteria are simply covered completely by the cold staining fluid.

The cover-glass or slide, with the charged side uppermost, may either rest on the table or be held by some modification of Cornet's forceps. When the solution is to be warmed the cover-glass may be floated, smeared side down, upon the fluid contained in a porcelain dish resting on a wire mat, supported on a stand, or it may be held in the Cornet forceps. If a slide is used it is simply inserted in the fluid or covered by it. The fluid in both the dish and on the glass should be carefully warmed, so as to steam without actually boiling. The glass should be kept completely covered with fluid. The bacteria having now been stained, the cover-glass or slide is grasped in the forceps and thoroughly but gently washed in clean water and then dried, first between layers of filter-paper and then in the air or high over a flame. A drop of balsam or water is now placed on a glass slide and the cover-glass placed upon it with the bacterial side down. The cover-glass or slide preparation is now ready for microscopic examination.

Staining of Bacteria.—The protoplasm of bacteria reacts to stains much as nuclear chromatin, though sometimes more and sometimes less actively.

The best stains are the basic aniline dyes, which are compounds derived from the coal-tar product aniline ($C_6H_5NH_2$).

ANILINE DYES.—The aniline dyes which are employed for staining purposes are divided into two groups according as the staining action depends on the basic or the acid portion of the molecule. The former contains amido groups and are spoken of as nuclear stains, since they color the nuclear chromatin of both cells and bacteria. The latter contain hydroxyl groups and do not stain bacteria, but are used chiefly for contrast coloring. The basic dyes are usually employed as salts of hydrochloric acid, while the acid dyes occur as sodium or potassium salts.

The following are the most commonly used basic aniline stains:

Violet stains—methyl violet, gentian violet, crystal violet.
Blue stains—methylene blue, thionin blue.
Red stains—basic fuchsin, safranin.
Brown stain—Bismarck brown.
Green stain—methyl green.

Of the above stains the violet and red stains are the most intense in action. It is thus easy to overstain a specimen with them. Of the blue, methylene-blue probably gives the best differentiation of structure and it is difficult to overstain with it.

These dyes are all more or less crystalline powders, and while some are definite chemical compounds, others are mixtures. For this reason various brands are met with on the market and the exact duplication of stains is not always possible. Dyes should be obtained from reliable houses only; most bacteriologists obtain them from Grübler, of Leipzig.

It is advisable to keep on hand not only the important dyes, but also stock solutions from which the staining solutions are made. The stock saturated alcoholic solutions are made by pouring into a bottle enough of the dye in substance to fill them to about one-quarter of their capacity. The bottle should then be filled with alcohol, tightly corked, well shaken, and allowed to stand for twenty-four hours. If at the end of this time all the staining material has been dissolved, more should be added, the bottle being again shaken and allowed to stand for another twenty-four hours. This must be repeated until a permanent sediment of undissolved coloring matter is seen upon the bottom of the bottle. This will then be labelled "saturated alcoholic solution," of whatever dye has been employed. The alcoholic solutions are not themselves employed for staining purposes. The solution for use is made by filling a small bottle three-fourths with distilled water, and then adding the concentrated alcoholic solution of the dye, little by little, until one can just see through the solution. Care must be taken that the color does not become too dense; usually about one part to ten is sufficient. Small wooden cases come prepared for holding about one-half dozen bottles of the staining solutions. This number will answer for all purposes.

General Observations on the Principles of Staining Bacteria.

Microchemical Reaction and Staining of the Cell Body.—Of special importance in this regard is the resistance which bacteria possess to diluted alkalies. Inasmuch as the majority of animal tissues are dissolved when treated with alkalies, this method has been adopted for rendering visible unstained bacteria in tissues. As a rule, bacteria are stained yellowish with iodine solution, a few only in consequence of their starchy constituents being stained blue.

Bacteria may be stained with various dyes of very different chemical composition, such as hæmatoxylin and certain plant dyes, etc., but most of these are of little practical value as compared with the basic aniline colors. R. Koch was the first to recognize the affinity of bacteria

for these dyes and to note their importance as a means of differentiating micro-organisms from other corpuscular elements.

The staining of bacteria is not to be considered simply as a mechanical saturation of the cell body with the dye, in which the latter is dissolved in the plasma. It is rather a chemical combination between the dye substance and the plasma. This union, however, is apparently an unstable one and easily broken up. Unna believes that the basic aniline dyes, from their chemical composition, are not really bases but neutral salts—*e. g.*, fuchsin equals rosaniline chloride; they are called basic only because the staining components (as the rosaniline) are of a basic nature. The staining process is, therefore, not to be looked upon as if the dye substance separated into its component parts and only the staining ingredient attacked the cell body, because the tissues for which these "basic aniline dyes" have special affinity are themselves basic. On the contrary, the dyestuff unites as a whole with the plasma, forming, as it were, a double salt or unstable compound between the two.

The dependence of the staining process upon the solvent condition of the dye is shown in the following observations:

1. Entirely water free, pure alcoholic dye solutions do not stain.
2. Absolute alcohol does not decolorize bacteria, while diluted alcohol is an active decolorizing agent. The compound of dye substance and plasma is therefore insoluble in pure alcohol.
3. The more completely a dye is dissolved the weaker is its staining power. For this reason pure alcoholic solutions are inactive; and the so-called weak dye solutions to which strong dye solvents have been added are limited in their action on certain bacteria in which the dye substance is closely united. This is the principle of Neisser's stain for diphtheria bacilli—viz., acetic acid methylene-blue solution.

On the other hand, the addition of alkalies to the dye mixture renders the solvent action less complete, leading to slight clouding, and finally, if large amounts of alkali are added, to precipitation of the dye substance. Solutions thus treated possess an intense staining power. According to Michaels, however, in Loeffler's methylene-blue solution the role of the alkali is purely of a chemical nature, by which it converts the methylene blue into methylene azure (azure II).

The dependence of the staining process upon the nature of the bacteria is exhibited in the following facts:

There are among bacteria some which are easily stained and others which are only stained with difficulty. To the latter belong, for example, the tubercle bacillus and lepra bacillus, also spores and flagella. The difference between them is that the easily stained objects require but a minimum of time to be immersed in a watery solution, while the others must be stained by special dyes or with the aid of outside influences (heat and mordants, etc.). The difficultly stained objects are at the same time not easily decolorized. The explanation of the resistance which these bacteria show to staining as well as to decolorizing agents is to be sought in two ways: either on the assumption that they possess a difficultly permeable or resisting envelope, or that they have

a special chemical constitution. The latter hypothesis holds good only, if at all, in regard to flagella and spores; while the assumption of the resisting envelope has reference more particularly to the tubercle bacillus, and is probably correct. The presence of fatty and waxy bodies in the envelope of these micro-organisms is capable of demonstration. Moreover, after extraction of these bodies by ether the tubercle bacillus loses its power of resisting acids, which peculiar resistance can also be artificially produced in other bacteria having normally no such resisting power. In many instances, doubtless, both of these causes, viz., resistant envelope and chemically different constitution, work together to produce the above-mentioned results.

Individual differences in acid resistance among the difficultly stained bacteria have been observed in tubercle bacilli; according to Ziehl and Ehrlich those having less individual resistance are probably the younger members. Individual differences in staining, in the easily stained bacteria, have also been noticed; for example, cholera vibrios and allied species are best stained with fuchsin, not so well with methylene blue, etc.

The relation between staining and degeneration of bacteria is a complicated question. Decrease of staining power takes place during degeneration of the bacterial cell, but it is often difficult to determine the exact moment when this loss of power occurs. Degenerated forms of the cholera bacillus from the abdominal cavity of guinea-pigs thus soon lose their power of staining in methylene-blue solution, but stain well in diluted carbol fuchsin. Moreover, bacteria killed by drying and moderate heating, as in the preparation of films, retain their power of staining. Kitasato found dead tubercle bacilli in sputum which took on normal staining. Bacteria killed by chloroform, formalin, etc., still retain their staining properties intact.

Elective staining properties, whereby certain species of bacteria are exclusively or rapidly and intensely stained by certain dyes, have repeatedly been observed. Of the greatest practical importance in this respect is the Gram stain used for the differential diagnosis of many species of bacteria; although a distinct classification of bacteria into those which are stained and those which are not stained by Gram's solution has been shown to be impracticable. There are some bacteria, however, which act uniformly toward Gram under all conditions; as, for example, the anthrax bacillus and the pyogenic cocci are always positive, the cholera and plague bacilli and gonococci are always negative to Gram. Other species again are at one time stained and at another decolorized by Gram; thus pyocyaneus is stained only in young individuals. Previous heating or extraction with ether, according to Nikitine, does not prevent the action of Gram's stain, but treatment with acids or alkalies renders it impossible. Bacteria so treated, however, after one hour's immersion in Loeffler's mordant regain their property of staining with Gram.

As to the nature of Gram's staining solution it may be mentioned that only the pararosanilines (gentian violet, methyl violet, and Victoria blue) are suitable for the purpose, whereas the rosanilines (fuchsin

and methylene blue) give negative results. The reason for this is that the iodine compounds with the pararosanilines are fast colors, while those with the rosanilines are unstable. These latter compounds when treated with alcohol break up into their constituents, the iodine is washed out, and the dye substance remaining in the tissues stain them uniformly, that is, without differentiation. But iodine-pararosaniline compounds are not thus broken up and consequently stain those portions of the tissue more or less, according to the affinity which they have for the dye substance. The parts stained by Gram are thus distinguished from those stained violet, not only quantitatively, but qualitatively; it is not a gentian violet but an iodine-rosaniline staining which occurs.

Use of Mordants and Decolorizing Agents.—In films of blood and pus, and in tissue sections, the tissue elements may be stained to such an extent as to obscure the bacteria. Hence many methods have been devised to use substances which, while increasing the staining power, tend to fix the stain in the bacteria and further to treat by substances which decolorize the overstained tissue to a greater or less extent while leaving the bacteria stained. The staining capacity of a solution may be increased by (*a*) the addition of substances such as carbolic acid, aniline oil, or metallic salts, all of which probably act as mordants; (*b*) by the addition of weak alkaline solutions of caustic potash or ammonium carbonate; (*c*) by the employment of heat; (*d*) by long duration of the staining process.

As decolorizing agents we use chiefly mineral acids (hydrochloric, nitric, sulphuric), vegetable acids (acetic), alcohol or a combination of alcohol and acid; also various oils, aniline, clove, etc.

The acid aniline dyes are represented by eosin, acid fuchsin, and fluorescein.

Formulæ of the Most Commonly Used Stain Combinations. LOEFFLER'S ALKALINE METHYLENE-BLUE SOLUTION. This consists of concentrated alcoholic solution of methylene blue, 30 c.c.; caustic potash in a 0.01 per cent. solution, 100 c.c. The alkali not only makes the cell more permeable, but also increases the staining power by liberating the free base from the dye.

KOCH–EHRLICH ANILINE-WATER SOLUTION OF FUCHSIN OR GENTIAN VIOLET is prepared as follows: To 98 c.c. of distilled water add 2 c.c. aniline oil, or more roughly but with equally good results, pour a few cubic centimetres of aniline oil into a test-tube, then add sufficient water to nearly fill it. In either case the mixtures are thoroughly shaken and then filtered into a beaker through moistened filter-paper until the filtrate is perfectly clear. To 75 c.c. of the filtrate add 25 c.c. of the concentrated alcoholic solution of either fuchsin, methylene blue, or gentian violet, or add the alcoholic solution until the aniline water becomes opaque and a film begins to form on the surface.

CARBOLIC FUCHSIN, OR ZIEHL'S SOLUTION.—Distilled water, 100 c.c.; carbolic acid (crystalline), 5 gm.; alcohol, 10 c.c.; fuchsin, 1 gm.; or it may be prepared by adding to a 5 per cent. watery solution of car-

bolic acid the saturated alcoholic solution of fuchsin until a metallic lustre appears on the surface of the fluid. The carbolic acid, like the alkali, favors the penetration of the stain.

The last two methods, combined with heating, are used to stain spores and certain resistant bacteria intensely, so that they retain their color when exposed to decolorizing agents.

Carbolic methylene blue, first used by Kühne, consists of 1.5 gm. of methylene blue, 10 gm. of absolute alcohol, and 100 c.c. of a 5 per cent. solution of carbolic acid. Carbolic thionin consists of 10 parts of a saturated alcoholic solution of thionin and 100 parts of a 1 per cent. solution of carbolic acid.

GRAM'S STAIN.—Another differential method of staining which is employed is that known as Gram's method. In this method the objects to be stained are floated on or covered with the aniline or carbolic gentian-violet solution. After remaining in this for a few minutes they are rinsed in water and then immersed in an iodine solution (Lugol's), composed of iodine, 1 gm.; potassium iodide, 2 gm.; distilled water, 300 c.c. In this they remain for from one to three minutes and are again rinsed in water. They are then placed in strong alcohol until most of the dye has been washed out. If the cover-glass as a whole still shows a violet color, it is again treated with the iodine solution, followed by alcohol, and this is continued until no trace of violet color is visible to the naked eye. They may then be washed in water and examined, or a contrasting color of eosin, fuchsin, carmine, or Bismarck brown may be given them by inserting them in weak solutions of these dyes for a few minutes. This method is useful in demonstrating the capsule which is seen to surround some bacteria—particularly the pneumococcus—and also in differentiating between varieties of bacteria, for some do and others do not retain their stain when put in the iodine solution for a suitable time.

Staining of Capsules.—Many methods of demonstrating the capsule have deen devised. Two only will be given here. The glacial acetic acid method, as described by Welch, is as follows: 1. Cover the preparation with glacial acetic acid for a few seconds. 2. Drain off and replace with aniline gentian-violet solution; this is to be repeatedly added until all the acid is replaced. 3. Wash in 1 to 2 per cent. solution of sodium chloride and mount in the same.

HISS' COPPER SULPHATE METHOD.—The organisms are grown, if possible, on ascitic fluid or serum media. If not, spread the organisms on the cover-glass by mixing with a drop of serum, or, better, a drop of one of the diluted serum media. Dry in the air and fix by heat.

The capsules are stained by the following method: A 5 per cent. or 10 per cent. aqueous solution of gentian violet or fuchsin (5 c.c. saturated alcoholic solution gentian violet to 95 c.c. distilled water) is used. This is placed on the dried and fixed cover-glass preparation and gently heated for a few seconds until steam arises. The dye is washed off with a 20 per cent. solution of copper sulphate (crystals). The preparation is then placed between filter-paper and thoroughly dried.

Staining Spores.—We have already noted that during certain stages in the growth of a number of bacteria spores are formed which refuse to take up color when the bacteria are stained in the ordinary manner. Special methods have been devised for causing the color to penetrate through the resistant spore. In the simplest method a cover-slip after having been prepared in the usual way is covered with Ziehl's carbolic fuchsin solution and held over the Bunsen flame until the fluid steams. This is continued for one or two minutes. It is then washed and dipped in a decolorizing acid solution, such as a 2 per cent. alcoholic solution of nitric acid, or a 1 per cent. solution of sulphuric acid in water, until all visible color has disappeared, then it is washed off and dipped for one-half minute in a saturated watery solution of methylene blue. The bacilli will then be blue and the spores red. Sometimes, however, the spores refuse to take the stain in

FIG. 52

Culture stain by Hiss' method.

this manner. We then can adopt Moeller's method, which is designed still further to favor the penetration of the coloring matter through the spore membrane. The prepared cover-slip is held for two minutes in chloroform, then washed off in water, then placed from one-half to three minutes in a 5 per cent. solution of chromic acid, again washed off in water, and now stained by adding to it carbolic fuchsin, which is steamed for several minutes. The staining fluid is then washed off and the preparation decolorized in a 3 per cent. solution of hydrochloric acid or a 5 per cent. solution of sulphuric acid. The preparation is finally stained for a minute in methylene-blue solution. The spores will be red and the body of the cells blue. The different spores vary greatly in the readiness with which they take up the dyes, and we have, therefore, to experiment with each variety as to the length of time they

should be exposed to the maceration of the chromic acid. Even under the best conditions it is almost impossible to stain some spores.

Staining Flagella.—For the demonstration of flagella, which are possessed by all motile bacteria, we are indebted to Loeffler. The staining of flagella satisfactorily is one of the most difficult of bacteriological procedures. Special stains devised by him, by Van Ermengem, by Pitfield, and others are employed.

Preparation of Films.—In all methods young (twelve to eighteen-hour) cultures on agar should be chosen. A little of the culture is carefully removed and placed in a few drops of filtered tap water. A tiny drop of this rather thin emulsion is allowed to spread with as little manipulation as possible over the cover-glass.

Bunge's modification of Loeffler's method is carried out as follows: Cover-glasses which have been most carefully cleaned are covered by a very thin smear. After drying in the air and passing three times through the flame the smear is treated with a mordant solution, which is prepared as follows: To 3 parts of saturated watery solution of tannin add 1 part of a 25 per cent. solution of ferric chloride. This mordant should be allowed to stand for several weeks before using. After preparing the cover-slip with all precautions necessary to cleanliness the filtered mordant is allowed to act cold for five minutes, after which it is warmed and then in one minute washed off. After drying, the smear is stained with the carbol-fuchsin solution or carbol-gentian violet, and then washed off, dried, and mounted.

Frequently the flagella appear well stained, but often the process has to be repeated a number of times. Overheating of the film prevents the staining of the flagella.

Van Ermengem's method gives good results.

Examination of Bacteria in Tissues.—Occasionally it is of importance to examine the bacteria as they are in the tissues themselves. The tissues should be obtained soon after death, so as to prevent as much as possible post-mortem changes, with consequent increase or decrease in the number of bacteria in them. Selected pieces of tissues can be frozen by ether and sections cut, but the best results are obtained when the material is embedded in celloidin or, better still, in paraffin. From the properly selected spots small portions, not thicker than one-quarter of an inch, are removed and placed in absolute alcohol for one or two days if less than one-eighth inch thick, and longer if thicker. For the larger pieces it is better to change the aclohol after twenty-four hours. The tissue pieces should be kept from falling to the bottom as the higher layers of alcohol remain nearer absolute. If along with the bacteria one wishes to study the finer structure of the tissue we must employ formalin or corrosive sublimate. The tissue is put in formalin 4 to 10 per cent. solution for three to twenty-four hours, and then in alcohol. Corrosive sublimate as a saturated solution in 0.75 per cent. sodium chloride solution is also an excellent fixative agent. Dissolve the sublimate in the salt solution by heat and then allow to cool, when the separation of crystals will show that saturation is complete. For pieces of tissue one-eighth inch

in thickness twelve hours' immersion is sufficient, if larger twenty-four hours is necessary. They should then be placed in pieces of gauze and left in running water for from twelve to twenty-four hours, according to the size of the pieces, to wash out the excess of sublimate. They are then placed for twenty-four hours in each of the following strengths of methylated spirit (free from naphtha): 30 per cent., 60 per cent., and 90 per cent. Finally they are placed in absolute alcohol for twenty-four hours and are then ready to be prepared for cutting according to the usual histological methods. The paraffin sections of tissue having been prepared and cut, they are ready for staining.

LOEFFLER'S METHOD.—The section is placed in Loeffler's alkaline methylene-blue solution for 5 to 30 minutes, then placed for a few seconds in 1 per cent. acetic acid. It is then placed in absolute alcohol, xylol, and Canada balsam. The number of seconds during which the preparation remains in the acetic acid must be tested by trials. The bacteria are dark blue, the nuclei blue, and the cell bodies light blue.

Thionin solution, carbol-fuchsin solution, and gentian violet can be used instead of Loeffler's methylene blue. Gram's method, with 3 per cent. hydrochloric acid in alcohol as a tissue decolorizer for ten seconds, is also valuable.

Preservation of Specimens.—Dry unstained or stained preparations of bacteria keep indefinitely, but if mounted in Canada balsam, cedar oil, or dammar lac they tend to gradually fade, but may be preserved for many months or years.

THE MICROSCOPE AND THE MICROSCOPIC EXAMINATION OF BACTERIA.

Different Parts of the Microscope.—A complete instrument usually has four oculars, or eye-pieces (*A*), which are numbered from 1 to 4, according to the amount of magnification which they yield. Numbers 2 and 4 are most useful for bacteriological work. The objective—the lens at the distal end of the barrel—serves to give the main magnification of the object. For stained bacteria the $\frac{1}{12}$ achromatic oil-immersion lens is regularly employed; except for photographic purposes the apochromatic lenses are not needed. Even here they are not indispensable. A $\frac{1}{16}$ may at times be useful, but hardly necessary; a No. 4 ocular and a $\frac{1}{12}$ lens give a magnification of about 1000 diameters (Fig. 55). For unstained bacteria we employ either the $\frac{1}{12}$ immersion or $\frac{1}{7}$ dry lens, according to the purpose for which we study the bacteria; for the examination of colonies where, as a rule, we do not wish to see individual bacteria, but only the general appearance of whole groups, we use lenses of much lower magnification (Fig. 56).

The stage *C*—the platform upon which the object rests—should be large enough to support the Petri plates if culture work is to be done. The iris diaphragm *D*, which is now regularly used in bacteriological work, opens and closes like the iris of the eye, and so controls the

amount of light. Its opening is diminished or increased by moving a small arm, which is underneath the stage, in one or another direction. The reflector placed beneath the stage serves to direct the light to the object to be examined. It has two surfaces—one concave and one convex. The coarse adjustment *F* is the rack-and-pinion arrangement by which the barrel of the microscope can be quickly raised or lowered. It is used to bring the bacteria roughly into focus. If the

FIG. 53

G

Microscope.

FIG. 54

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lens
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Tube
Tube
Tube Diaphragm
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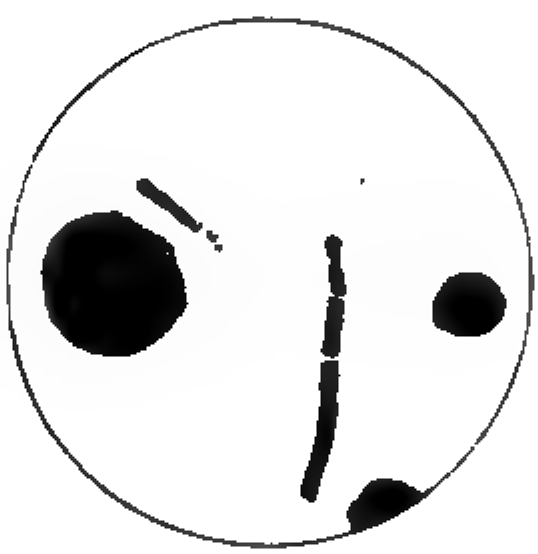
Distance
of the Objective
Object

Internal structure of the microscope. (After Spencer Lens Co.)

bearings become loose tighten the little screws at the back of the pinion box. Keep the teeth clean. If the bearings need oiling use an acid-free lubricant, such as paraffin oil. The fine adjustment *G* serves to raise and lower the barrel very slowly and evenly, and is used for the exact study of the bacteria when high-power lenses are used. It is necessarily of limited range and delicate in its mechanism. If, when look-

ing into the eye-piece, no change of focus is noticed by turning the micrometer head, or if the micrometer head ceases to turn, the adjustment has reached its limit. Turn the micrometer back to bring the fine adjustment midway within its range. When the fine adjustment head stops do not force it. For the microscopic study of bacteria it is essential that we magnify the bacteria as much as possible and still have their definition clear and sharp. It is essential, therefore, that the microscope be provided with an oil-immersion system and a substage condensing apparatus. In using the oil-immersion lens a drop of oil of the same index of refraction as the glass is placed upon the face of the lens, so as to connect it with the cover-glass when the bacteria are in focus. There is thus no loss of sight through deflection, as is the case in the dry system. If the lenses become dirty they should be wiped gently with Japanese lens paper or a clean, soft, old-linen handkerchief. If necessary breathe on the lens before wiping, and if

Fig. 55



Anthrax bacilli and blood cells. × 1000 diameters.

Fig. 56

Colonies of diphtheria bacilli. × 200 diameters.

this does not succeed use a little xylol or chloroform. These substances are not to be used unless necessary. An immersion objective should always be cleaned immediately after using. The objective should always be kept covered so as to prevent dust dropping in.

Light.—The best light is obtained from white clouds or a blue sky. Avoid direct sunlight. If necessary use white shades to modify the sunlight. Artificial light has one advantage over daylight in that it is constant in quality and quantity. The Welsbach burner and a whitened incandescent bulb give a good light. A blue glass between the artificial light and the lens is often of value. An eye-shade is often helpful.

Substage Condensing Apparatus *H* is a system of lenses situated beneath the central opening of the stage. It serves to condense the light passing through the reflector to the object in such a way that it is focused upon the object, thus furnishing the greatest amount of luminosity. Between the condenser and the reflector is placed an adjustable

diaphragm, the aperture of which can be regulated, as circumstances require, to permit of either a very small or a very large amount of light passing to the object.

Focusing.—Focus the body tube down by means of the coarse adjustment until the objective nearly touches the cover-glass, being careful not to touch it. Then with the eye at the eye-piece focus up carefully with the coarse adjustment until the specimen comes plainly into view. Be careful not to pass by this focal point without noticing it. This is likely to occur if the light be too intense and the specimen thin and transparent. If the sliding tube coarse adjustment is used, focus carefully by giving the tube a spiral movement.

When the object is brought fairly well into focus by means of the coarse adjustment, use the fine adjustment to focus on the particular spot desired, for if this spot is in the centre of the field of the low power it will be somewhere in the field of the higher power. It is too much to ask of the maker that the lenses be made absolutely parfocal and centred. The delicacy of the centring can be appreciated when the magnification and the extremely small portion examined are considered. When the objectives are not thus fitted to the nose-piece, refocusing and again hunting up the object are necessary. In so doing we repeat the caution to always focus up before turning the nose-piece. When no revolving nose-piece is used the change of objectives means the unscrewing of one and the screwing of the other into its place, and refocusing.

The beginner should always use the low-power objectives and oculars first. The low-power objectives have longer working distances and are not so apt to be injured. They always show a larger portion of the specimen and thus give one a better idea of the general contour. After obtaining this general idea the higher powers can be used to bring out greater detail in any particular part.

Tube Length and Cover-glass.—All objectives are corrected to a certain tube length (160 mm. by most makers—Leitz, 170 mm.) and all objectives in fixed mounts of over 0.70 N. A. are corrected to a definite thickness of cover-glass as well (Zeiss, 0.15 mm., 0.20 mm.; Leitz, 0.17 mm.; Bausch & Lomb and Spencer, 0.18 mm.). These objectives give their best results only when used with the cover-glass and tube length for which they are corrected. As indicated in Fig. 53 the tube length extends from the eye lens of the eye-piece to the end of the tube into which the objective or nose-piece is screwed. If a nose-piece is used the draw tube must be correspondingly shortened. If the cover-glass is thinner than that for which the objective is corrected, the tube must be lengthened to obtain best results; if thicker, shortened.

The more expensive objectives are provided with adjustable mounts by which the distances between the lens systems may be changed to compensate for difference of thickness of cover. They are successfully used only in the hands of an expert. One of them out of adjustment is worse than an ordinary objective.

Examination of Bacteria in the Hanging Drop.—It is often valuable to observe bacteria alive, so as to study them under natural conditions. We can thus note the method and rate of their multiplication, the presence or absence of spore formation, and their motility in fluids and their agglutination with specific serums. For this, special slides and methods are desirable. The usual form is one in which there is ground out on one surface a hollow having a diameter of about half an inch (Fig. 57). According to the purpose for which the hanging drop is to

FIG. 57

Hollow slide with cover-glass.

be studied, sterilization of the slide and cover-glass may or may not be necessary. The hanging block has already been described. The technique of preparing and studying the hanging drop is as follows: The surface of the glass around the hollow in the slide is smeared with a little vaselin or other inert substance. This has for its purpose both the sticking of the cover-glass to the slide and the prevention of evaporation in the drop placed in the little chamber, which is to be formed between the cover-glass when placed over the hollow, and the slide.

For the purpose of studying the bacteria we place, if they are in fluids, simply a platinum loopful upon the centre of the cover-glass and then invert it by means of a slender pair of forceps over the hollow in the slide, being very careful to have the bacteria over the very centre of the space. If the bacteria, on the contrary, are growing on solid media, or are obtained from thick pus or tissues from organs, they are mixed with a suitable amount of bouillon or sterile physiological salt solution either before or after being placed upon the cover-glass. If we wish to observe the bacteria under natural conditions we must keep the tiny drop of fluid at the proper temperature for the best growth of the bacteria. If, however, we simply wish to observe their form and arrangement this is not necessary.

In the study of living bacteria we often wish to observe their grouping and motion rather than their individual characters, and so use less magnification than for stained bacteria. In studying unstained bacteria and tissues we shut off as large a portion of the light with our diaphragm as is compatible with distinct vision, and thus favor contrasts which appear as lights and shadows, due to the differences in light transmission of the different materials under examination. It is necessary to remember that they are seen with difficulty, and that we are very apt, unless extremely careful in focusing, to allow the lens to go too far, and so come upon the cover-glass, break it, destroy our preparation, and, if examining parasitic bacteria, infect the lens. This may be avoided by first finding the hanging drop with a low-power lens and thus exactly centre it. The lens of higher magnification is now

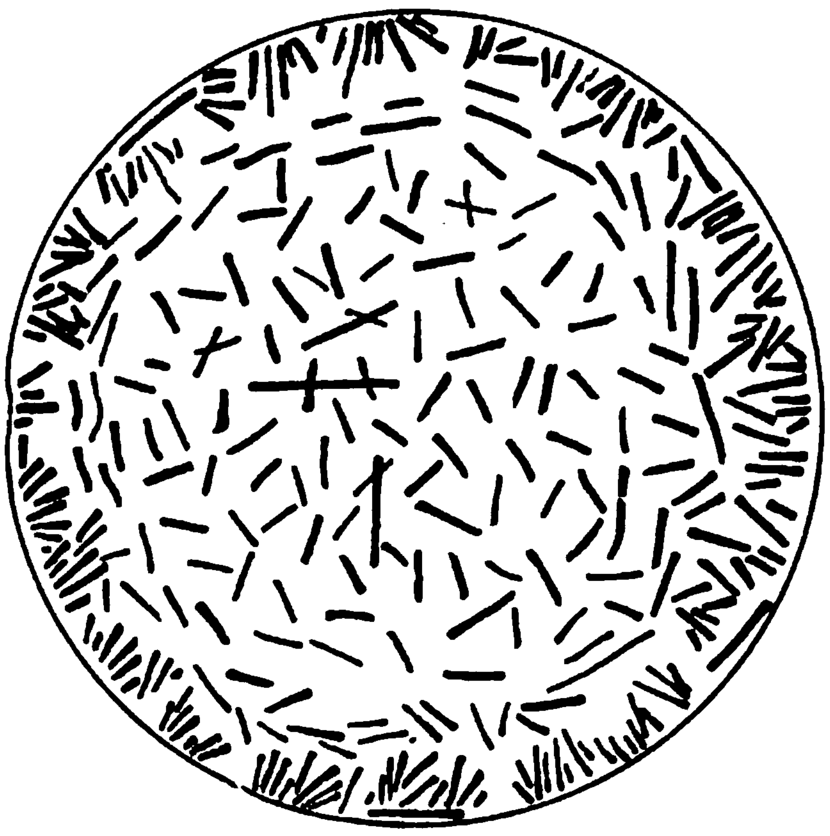
very gradually lowered, while at the same time gently moving the slide back and forth to the slightest extent possible with the left hand. If any resistance is felt the lens should be raised, for it has gone beyond the point of focus and is touching the cover-glass.

Testing Agglutinative Properties of Serum.—By agglutination is meant the aggregation into clumps of uniformly disposed bacteria in a fluid; by sedimentation the formation of a deposit composed of such clumps when the fluid is allowed to stand; sedimentation is thus the naked-eye evidence of agglutination.

The blood serum of animals is found to acquire the clumping power for almost every variety of motile bacteria, and for many non-motile forms after infection with that variety.

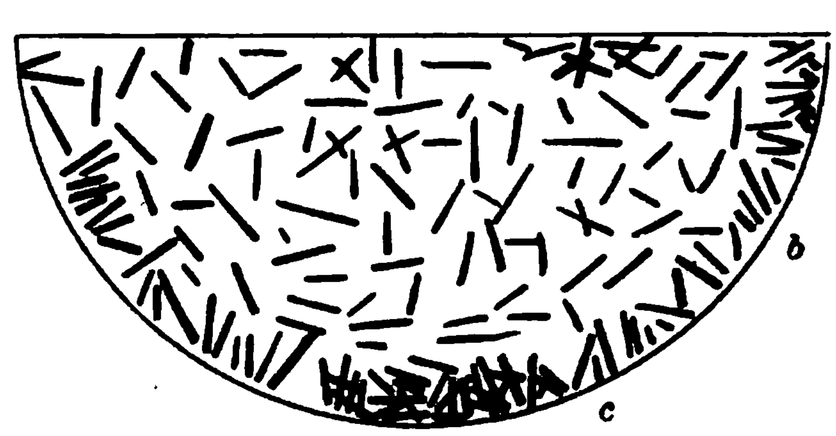
In order to help the student to thoroughly understand what comprises a reaction, Wilson prepared a set of drawings, which are here reproduced. The culture to be tested should be of about twenty hours'

FIG. 58



Microscopic field, showing the top of a hanging drop in a normal typhoid culture.

FIG. 59



Microscopic field, showing a cross-section of the drop in Fig. 58.

growth, either in bouillon or on agar. If on the latter a suspension is made in broth or normal salt solution. A loopful of the fluid containing the bacteria is placed on the cover-glass, and to it an equal quantity of the serum dilution is added.

In making the hanging drop to be examined it is necessary to have it of such a depth that it will show at least three focal planes, otherwise the examination will be incomplete and unsatisfactory. The moist chamber must be well sealed by vaselin so as to prevent drying, and kept at a temperature of at least 20° and not over 35° C.

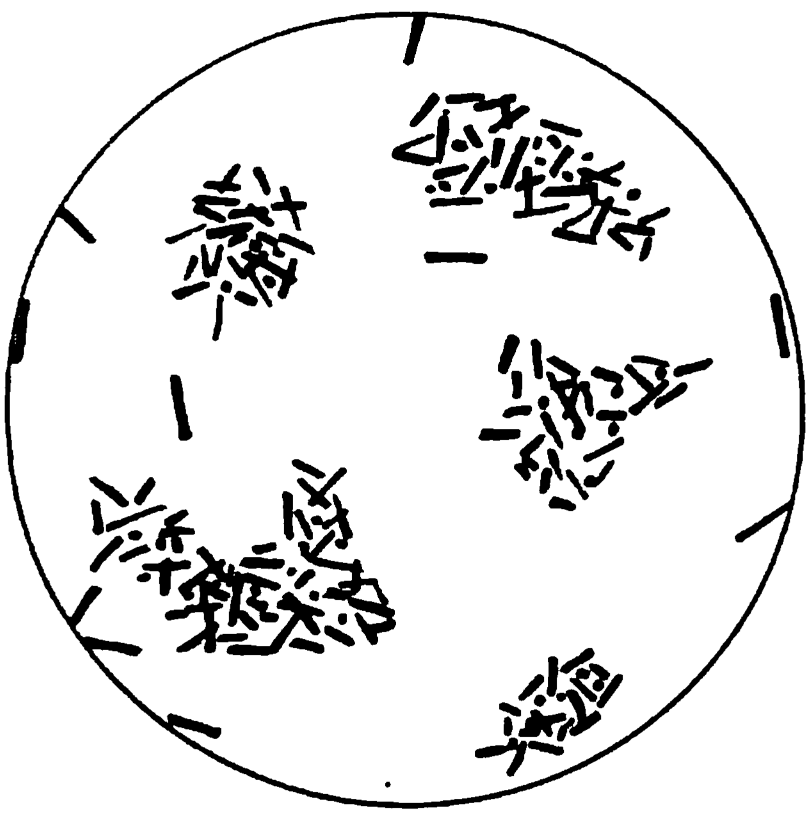
Fig. 58 shows a microscopic field of the *top* of a hanging drop of a normal bouillon culture of typhoid bacilli. The culture is twenty hours old and the organisms are freely motile. This represents the control drop used for comparison with the drop of the same culture,

to which has been added a little of the blood of a person suspected to have typhoid. Note these points in Fig. 58: the organisms are evenly distributed throughout the field, except at the edge of the drop, where they are gathered in great numbers; they show great activity here, seemingly trying to crowd to the very edge. This attraction is probably due to the action exerted on the organisms by the oxygen in the air, which naturally exerts positive chemotaxis on all aërobic organisms.

Fig. 59 shows a *cross-section* of the drop represented in Fig. 58, and it will be noticed that the bacilli are evenly distributed throughout the drop, except at one place in the focal plane *a*, and again in the focal plane *c*.

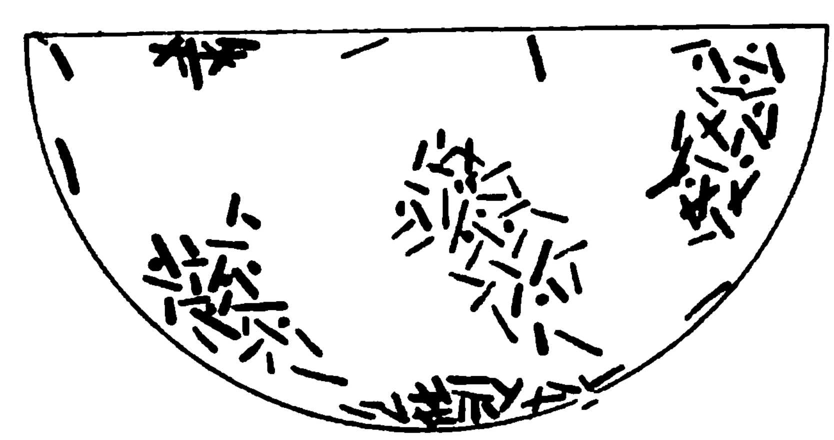
It sometimes happens that there is a substance adhering to a supposedly clean cover-glass which attracts the bacilli to that point, where

FIG. 60



Microscopic field, showing the top of a drop with the typhoid reaction.

FIG. 61



Microscopic field, showing a cross-section of the drop in Fig. 60.

they appear as fairly well-defined clumps, more or less like the true clumps due to the agglutinating substance in typhoid blood. The increase in organisms at the bottom of the drop in the focal plane *c* is easily accounted for by the fact that gravity naturally carries the dead and non-motile organisms to the bottom, these frequently assuming the character of clumps.

If a field can be found in any focal plane of the hanging drop free from clumps, one can be quite sure that any clumping present is not due to any agglutinating substance which necessarily will affect organisms in every focal plane.

Fig. 60 shows the microscopic appearance of the *top* of a drop where the reaction is present. Notice first that the organisms have been drawn together in groups and that the individuals of each group appear to be loosely held together. Viewed under the microscope these clumps are practically quiescent, there being very little movement either of the individual organisms or of the clump as a whole. The edge of the drop

is practically free from organisms, showing that the air no longer exerts any influence on them.

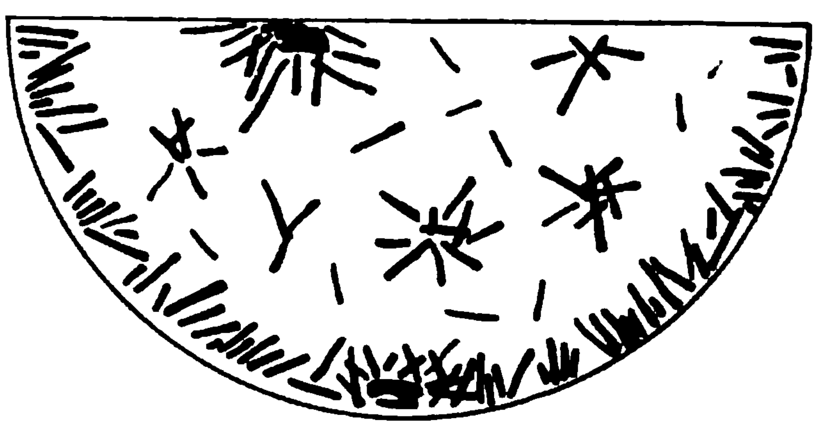
Fig. 61 shows a *cross-section* of the hanging drop shown in Fig. 60. The clumps are evenly distributed throughout the drop, with perhaps some increase in the numbers and compactness of the clumps at the bottom.

Fig. 62 shows the microscopic appearance of the *top* of a hanging drop of a bouillon culture to which has been added some blood of a patient suffering from a febrile condition not caused by typhoid infection, but which exerts a slight non-specific influence on the typhoid organisms. It will be seen that the reaction is incomplete and that there are many organisms at the edge of the drop. The air exerts the same influence on the bacilli that it did before the addition of the blood. Note the character of the clumps, generally small and compact at the centre, with the bacilli at the edge of the clump, usually attached by one end only.

Fig. 62

Microscopic field, showing the top of a drop of culture with reaction not due to typhoid.

Fig. 63



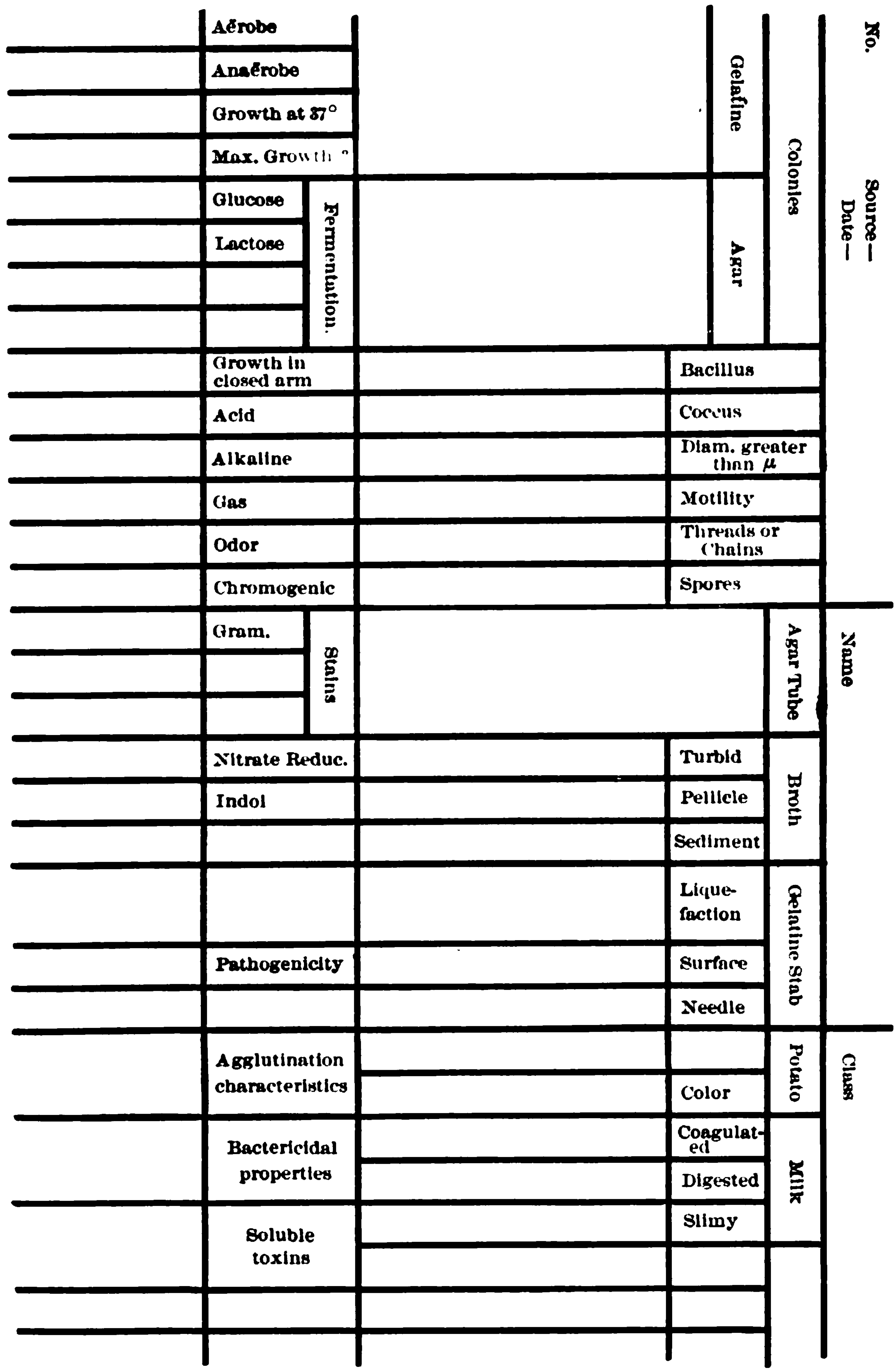
Microscopic field, showing a cross-section of Fig. 62.

Very frequently these clumps have the appearance of being built up around a piece of detritus present in the clump. All the organisms comprising the clump seem to have retained part, at least, of their motility, those on the edges being particularly motile, so far as their free ends are concerned.

When motility is very much inhibited these clumps have a peculiar trembling movement, which is like the molecular movement described by Brown.

Fig. 63 shows a cross-section of the drop represented in Fig. 62. Note the same character of the clumps in every focal plane: the large number of motile bacilli and the number attracted to the edge of the drop by the air.

Important Characteristics which should be Noted in the Complete Study of a Bacterium.—The accompanying chart gives the most important points to be investigated. With some varieties the cultural



Type of card used in studying characteristics of bacteria.

characteristics are of the greatest importance, while with others it is the study of pathogenic or toxic effects.

CHAPTER VII.

VITAL PHENOMENA OF BACTERIA—MOTION, HEAT, AND LIGHT PRODUCTION—CHEMICAL EFFECTS.

Motility.—Many bacteria when examined under the microscope are seen to exhibit active movements in fluids. The movements are of a varying character, being described as rotary, undulatory, sinuous, etc. At one time they may be slow and sluggish, at another so rapid that any detailed observation is impossible. Some bacteria are very active in their movements, different individuals progressing rapidly in different directions, while with many it is difficult to say positively whether there is any actual motility or whether the organism shows only molecular movements—so-called "Brownian" movements—a dancing, trembling motion possessed by all finely divided organic particles. Very young cultures in neutral nutrient bouillon should be examined at a temperature suitable for their best growth. Not all species of bacteria which have flagella exhibit at all times spontaneous movements; such movements may be absent in certain culture media and at too low or too high temperatures, or of either an insufficient or excessive supply of oxygen.

Chemotaxis.—Some chemical substances seem to exert a peculiar attraction for bacteria, known as *positive chemotaxis*, while others repel them—*negative chemotaxis*. Moreover, all varieties are not affected alike, for the same substances may exert on some bacteria an attraction and on others a repulsion. Oxygen, for example, attracts aërobic and repels anaërobic bacteria, and for each variety there is a definite proportion of oxygen, which most strongly attracts. The chemotactic properties of substances are tested by pushing the open end of a fine capillary tube, filled with the substance to be tested, into the edge of a drop of culture fluid containing bacteria and examining the hanging drop under the microscope. We are able thus to watch the action of the bacteria and note whether they crowd about the tube opening or are repelled from it. Among substances showing positive chemotaxis for nearly all bacteria are peptone and urea, while among those showing negative chemotaxis are alcohol and many of the metallic salts.

Production of Light.—Bacteria which have the property of emitting light are quite widely distributed in nature, particularly in media rich in salt, as in sea-water, salt fish, etc. Many of these, chiefly bacilli and spirilla, have been accurately studied. The emission of light is a property of the living protoplasm of the bacteria, and is not usually due to the oxidation of any photogenic substance given off by them; at least only in two instances has such substance been claimed to have been

isolated. Every agent which is injurious to the existence of the bacteria affects this property. Living bacteria are always found in phosphorescent cultures; a filtered culture free from germs is invariably non-phosphorescent; but while the organism cannot emit light except during life, it can live without emitting light, as in an atmosphere of carbonic acid gas, for instance. They are best grown under free access of oxygen in a culture medium prepared by boiling fish in sea-water (or water containing 3 per cent. sea-salt), to which 1 per cent. peptone, 1 per cent. glycerin, and 0.5 per cent. asparagin are added. Even in this medium the power of emitting light is soon lost unless the organism is constantly transplanted to fresh media.

Thermic Effects.—The production of heat by bacteria does not attract attention in our usual cultures because of its slight amount, and even fermenting culture liquids with abundance of bacteria cause no sensation of warmth when touched by the hand. Careful tests, however, show that heat is produced. The increase of temperature in organic substances when stored in a moist condition, as tobacco, hay, manure, etc., is one partly at least due to the action of bacteria.

Chemical Effects.—The processes which bodies being split up undergo depend, first, on the chemical nature of the bodies involved and the conditions under which they exist, and, secondly, on the varieties of bacteria present. A complete description of these chemical changes is at present impossible. Chemists can as yet only enumerate some of the final substances evolved, and describe, in a few cases, the manner in which they were produced. Bacteria are able to construct their body substance out of various kinds of nutrient materials and also to produce fermentation products or poisons, and they are able to do these things either analytically or synthetically with almost equal ease. This ambidextrous metabolic power exists, according to Hueppe, among bacteria to an extent known as yet among no other living things.

In the chemical building up of their body substance we can distinguish, as Hueppe concisely puts it, several groups of phenomena: Polymerization, a sort of doubling up of a simple compound; synthesis, a union of different kinds of simple compounds into one or more complex substances; formation of anhydride, by which new substances arise from a compound through the loss of water; and reduction or loss of oxygen, which is brought about especially by the entrance of hydrogen into the molecule. The breaking down of organic bodies of complicated molecular structure into simpler combinations takes place, on the other hand, through the loosening of the bonds of polymerization, through hydration or entrance of water into the molecule, and through oxidation.

The chemical effects which take place from the action of bacteria are greatly influenced by the presence or absence of free oxygen. The access of pure atmospheric oxygen makes the life processes of most bacteria more easy, but is not indispensable when available substances are present which can be broken up with sufficient ease. The standard of availability is very different for different bacteria.

In the presence of oxygen some of the decomposition products that are formed by the attack of the anaërobic bacteria are further decomposed and oxidized by the aërobes; they are thereby rendered, as a rule, inert and consequently harmless. Some bacteria have adapted themselves to the exclusive use of compound oxygen, using those compounds from which oxygen can be obtained, and others—the obligatory anaërobes—are able to live only in the presence of free oxygen. The facts of anaërobiosis are of great importance to technical biology and to pathology. Many parasitic bacteria are found to produce far more poison in the absence of air than in its presence. The following three types of chemical activity can be separated: 1. The bacteria develop their tissues. 2. The bacteria produce and liberate ferments or enzymes which tend to make the foodstuff in their neighborhood more assimilable. 3. The bacteria assimilate substance and liberate it changed to other material. These changes may be due to ferments retained in the cells.

Fermentation.—The term fermentation is differently used by different authors. Some call every kind of decomposition due to bacteria or their products a fermentation, speaking thus of the putrefactive fermentation of albuminous substances; others limit the term to the process when accompanied by the visible production of gas; others, again, take fermentation to mean only the decomposition of carbohydrates, with or without gas-production.

Fermentation may be defined as a chemical decomposition of an organic compound, induced by the life processes of living organisms (organized ferments), or by chemical substances thrown off from the bacteria (unorganized or chemical ferments or enzymes). In the first the action is due to the life processes necessary for the growth of the organisms producing the ferment, as in the formation of acetic acid from alcohol by the action of the vinegar plant, and in the second the enzyme, either within or outside of the organism and having no direct connection with the growth of the organism, causes a structural change without losing its identity, as in digestion. E. Buchner (*Berichte d. Deutsch. chem. Gesellsch.*, xxx. 117–124 and 1110–1113) has shown that, even in those cases of fermentation in which formerly it was believed the organized cell itself was necessarily concerned, the cell protoplasm squeezed from its capsule is able to cause the same changes as the organized cells. This brings fermentation by unorganized and organized ferments very closely together, the one being a substance thrown off from the cell, the other a substance ordinarily retained in the cell. The increase of both ceases with the death of the bacteria producing them. These enzymes, even when present in the most minute quantities, have the power of splitting up or decomposing complex organic compounds into simpler, more easily soluble and diffusible molecules. The changes thus made may greatly aid in rendering the foodstuff suitable for bacterial growth. We can only speak of chemical ferments when it can be demonstrated that the fermentation continues in the absence of all living bacteria. This

may be accomplished by the addition of disinfectants—carbolic acid, chloroform, ether, etc.—to the cultures or by filtration.

CHARACTERISTICS OF FERMENTS.—Ferments are non-dialyzable. They withstand moderate dry heat, but are usually destroyed in watery solutions by a temperature of over 70° C. They are injured by acids, especially the *inorganic* ones, but are resistant to all alkalies. A simple example of bacterial fermentation of carbohydrates produced by an enzyme is that of grape-sugar:

$$\underset{\text{Grape-sugar.}}{C_6H_{12}O_6} = \underset{\text{2 Alcohol.}}{2C_2H_6O} + \underset{\text{2 Carbon dioxide.}}{2CO_2}$$

Or,

$$\underset{\text{Grape-sugar.}}{C_6H_{12}O_6} = \underset{\text{2 Lactic acid.}}{2C_3H_6O_3}$$

Or,

$$\underset{\text{Grape-sugar.}}{C_6H_{12}O_6} = \underset{\text{3 Acetic acid.}}{3C_2H_4O_2}$$

Far less common is oxidizing fermentation, as in the production of acetic acid from alcohol. Here the energy is acquired not by the decomposition but by the oxidation of the alcohol.

The proteolytic or peptonizing ferments which are somewhat analogous to trypsin—being capable of changing albuminous bodies into soluble and diffusible substances—are very widely distributed. The liquefaction of gelatin, which is chemically allied to albumin, is due to the presence of a proteolytic ferment or trypsin. The production of proteolytic ferments by different cultures of the same varieties of bacteria varies considerably—far more than is generally supposed. Even among the freely liquefying bacteria, such as the cholera spirillum and the staphylococcus, poorly liquefying varieties have been repeatedly found. These observations have detracted considerably from the value in cultures of the property of liquefying gelatin as a positive diagnostic characteristic. Most conditions which are unfavorable to the growth of bacteria seem to interfere also with their liquefying power.

Certain bitter-tasting products of decomposition are formed by liquefying bacteria in media containing proteid, as, for example, in milk.

Diastatic ferments convert starch into sugar. That these are produced by bacteria is shown by mixing starch paste with cultures to the resulting mixture of which thymol has been added, and keeping the digestion for six to eight hours in the incubating oven; then, on the addition of Fehling's solution and heating, the reaction for sugar appears —the reddish-yellow precipitate due to the reduction of the copper. Bacteria may be directly tested for sugar also by boiling potato-broth cultures and using the extract.

Inverting ferments (that is, those which convert polysaccharides into monosaccharides) are of very frequent occurrence. Bacterial invertin withstands a temperature of 100° C. for more than an hour, and is produced in culture media free from proteid. For more details as to the action of ferments on sugars see chapter on the colon-typhoid groups.

Rennin-like ferments (substances having the power of coagulating milk with neutral reaction, independent of acids) are found not infrequently among bacteria. The B. prodigiosus, for instance, in from one to two days coagulates to a solid mass milk which has been sterilized at 55° to 60° C. These ferments have not been thoroughly investigated; they are probably present, however, in all species of bacteria which coagulate milk, even though the organisms also produce acid.

Fermentation yields products that are poisonous to the ferment; hence fermentation ceases when the nutriment is exhausted or the fermentation is in excess. Different kinds of fermentation obtain specific names, according to the products. Thus *acetic*, yielding acetic acid; *alcoholic* or *vinous*, yielding alcohol; *ammoniacal*, yielding ammonia; *amylic*, yielding amylic alcohol; *benzoic*, yielding benzoic acid; *butyric*, yielding butyric acid; *lactic*, yielding lactic acid; and *viscous*, yielding a gummy mass.

Pigment Production.—Pigments have no known importance in connection with disease, but are of interest and have value in identifying bacteria. Their chemical composition is not generally known.

RED AND YELLOW PIGMENTS.—Of the twenty-seven red and yellow bacteria studied by Schneider, almost all produce pigments soluble in alcohol and insoluble in water. The larger majority of these possess in common the property of being colored blue-green by sulphuric acid and red or orange by a solution of potash. Though varying considerably in their chemical composition and in their spectra, they may be classified, for the most part, among that large group of pigments common to both the animal and vegetable kingdoms known as *lipochromes*, and to which belong the pigments of fat, yolk of egg, the carotin of carrots, turnips, etc.

VIOLET PIGMENTS.—Certain bacteria produce violet pigments, also insoluble in water and soluble in alcohol, but insoluble in ether, benzol, and chloroform. These are colored yellow when treated in a dry state with sulphuric acid and emerald-green with potash solution.

BLUE PIGMENTS, such as the blue pyocyanin, are also produced by the so-called fluorescent bacteria, along with a pigment named bacteriofluorescin. In cultures the fluorescence is at first blue; later, as the cultures become alkaline it is green.

Numerous investigations have been made to determine the cause of the variation in the chromogenic function of bacteria. All conditions which are unfavorable to the growth of the bacteria decrease the production of pigment, as cultivation in unsuitable media or at too low or too high a temperature, etc. The B. prodigiosus produces no pigment at 37° C., and when transplanted at this temperature, even into favorable media, the power of pigment production is gradually lost. B. pyocyaneus does not produce pigment under anaërobic conditions.

Ordinarily colorless species of bacteria sometimes produce pigments. Thus yellow colonies of the pneumococcus have been observed, and colored varieties of the streptococcus pyogenes. Occasionally colored and uncolored colonies of the same species of bacteria may be seen to

occur side by side in one plate culture, as, for example, the staphylococcus pyogenes.

Alkaline Products and the Fermentation of Urea.—Aërobic bacteria always produce alkaline products from albuminous substances in culture media free from sugar. Many species of bacteria produce acids in the presence of sugars, which explains the fact that neutral or slightly alkaline broth often becomes acid at first from the fermentation of the sugar contained in the meat used for making the media. When the sugar is used up the reaction often becomes alkaline, as the production of alkalies continues. The substances producing the alkalinity in cultures are chiefly ammonia, amine, and the ammonium bases.

The conversion of urea into carbonate of ammonia affords an example of the production of alkaline substances by bacteria:

$$\underset{\text{Urea.}}{CO(NH_2)_2} \quad + \quad \underset{\text{2 Water.}}{2H_2O} \quad = \quad \underset{\text{Ammonium carbonate.}}{CO_3(NH_4)_2}$$

The power of decomposing urea is not widespread among bacteria. Of sixty species investigated by Lehman, three only developed the odor of ammonia from sterilized human urine.

Ptomains.—Nencki, and then later Brieger, Vaughan, and others, succeeded in preparing organic bases of a definite chemical composition out of putrefying fluids—meat, fish, old cheese, and milk—as well as from pure bacterial cultures. Some of these were found to exert a poisonous effect, while others were harmless. The poisons may be present in the decomposing cadaver (hence the name ptomain, from *πτῶμα*, putrefaction), and, in consequence, have to be taken into consideration in questions of legal medicine. They may be formed also in the living human body, and, if not made harmless by oxidation, may come to act therein as self-poisons or leucomains. They are now known not to be the substances which excite the specific poisonous effects of bacteria. The latter are easily destroyed by heat, and have entirely different characteristics.

Many ptomains are known already and the elementary composition of each made out, and among them are some whose exact chemical constitution is established. Especially interesting is the substance cadaverin, which was separated by Brieger from portions of decomposing dead bodies and from cholera cultures, by reason of the fact that Ladenburg prepared it synthetically and showed it to be pentamethylenediamin [$(NH_2)_2(CH_2)_5$]. The cholin group is particularly interesting. Cholin itself ($C_5H_{15}NO_2$) arises from the hydrolytic breaking-up of lecithin, the fat-like substance found in the brain and other nervous tissue. By the oxidation of cholin there can be produced the highly toxic muscarin, found by Schmiedeberg in a poisonous toadstool and by Brieger in certain decomposing substances:

$$\underset{\text{Cholin.}}{C_5H_{16}NO_2} \quad + \quad O \quad = \quad \underset{\text{Muscarin.}}{C_5H_{15}NO_3}$$

The ptomain tyrotoxicon was obtained from cheese, milk, and ice-cream by Vaughan.

Pyocyanin ($C_{14}H_{14}N_2O$), which produces the color of blue or blue-green pus, is a ptomainic pigment. Similar bodies of a basic nature may be found in the intestinal contents as the products of bacterial decomposition. Some of these are poisons and can be absorbed into the body. Some believe the symptoms designated as coma and tetany may be ascribed to the absorption of substances of this nature. Since the name ptomain was given to the poisonous products of bacterial growth before these products were chemically understood it is by many wrongly applied to all poisons found in food, as in cases of poisoning due to decomposing meat or sausage or to cheese or milk. These are sometimes true toxins or even living bacteria.

The isolation of these substances can here be only briefly referred to. According to Brieger's method, which is the one now generally employed, the cultures having a slight acid reaction (HCl) are boiled down, filtered, and the filtrate concentrated to a syrupy consistency, dissolved in 96 per cent. alcohol, purified and precipitated by means of an alcoholic solution of bichloride of mercury.

THE MORE COMPLICATED PROTEID POISONS.

These are divided into bacterial proteins, toxins, and endotoxins.

Bacterial Proteins.—These substances are bacterial poisons which are little or not at all specific, that excite fever, inflammation, and suppuration, and resist the boiling temperature. They are usually extracted by boiling cultures in 0.5 per cent. sodium hydrate solution. The proteins are precipitated in weak acid solutions. *Tuberculin* is the most important of the group; *mallein* is another.

Like mallein, according to Buchner and Römer, all bacterial proteins are very similar in their action, and Matthes maintains that deutero-albumose, which is obtained by the action of pepsin on albumin and has no connection with bacteria, has an effect on tuberculous guinea-pigs somewhat similar to tuberculin.

Toxins.—Toxins are poisonous synthetical products of bacterial growth.

The exact composition of toxins has not as yet been discovered, but it is believed that they are of proteid character. At first all the toxins were supposed to be albumins, but recently some of the most important, such as those produced by the tetanus and diphtheria bacilli, have been shown to possess characteristics which separate them from that class. Toxins are formed during the growth of bacteria in media containing no proteid, but more abundantly when it is present. Toxins are divided into extracellular and intracellular poisons. Thus, the toxins produced by the diphtheria and tetanus bacilli during their growth in the tissues or culture media are largely given up to the culture fluid, but little remaining in the bacterial protoplasm, while the toxins elaborated by the typhoid, tubercle, glanders, and colon bacilli, and indeed by the majority of parasitic and saprophytic bacteria, are largely retained in the bodies of the bacteria until their death and destruction.

Among the properties of the extracellular toxins are the following: They are, so far as known, uncrystallizable, and thus differ from ptomains; they are soluble in water and they are dialyzable; they are precipitated along with proteids by concentrated alcohol, and also by ammonium sulphate; if they are proteids they are either albumoses or allied to the albumoses; they are relatively unstable, having their toxicity diminished or destroyed by heat (the degree of heat, etc., which is destructive varies much in different cases). Their potency is often altered in the precipitations practised to obtain them in a pure or concentrated condition, but among the precipitants ammonium sulphate has little if any harmful effect. Regarding the intracellular toxins which are more intimately associated with the bacterial cell we know much less, but it is probable that their nature is similar, though some of them at least are not so easily injured by heat—*e. g.*, in the case of the product of tubercle bacilli. In the case of all toxins the fatal dose for an animal varies directly with the species, body weight, age, and previous conditions as to, *e. g.*, food, temperature, etc. In estimating the minimal lethal dose of a toxin these factors must be carefully considered.

The following is the method usually employed for obtaining concentrated extracellular toxins. The toxic fluid is placed in a shallow dish, and ammonium sulphate crystals are well stirred in till no more dissolve. Fresh crystals to form a bulk nearly equal to that of the whole fluid are added, and the dish is set in an incubator at 37° C. (98.6° F.) overnight. Next day a brown scum of precipitate will be found floating on the surface. This contains the toxin. It is skimmed off with a spoon, placed in watch-glasses, and these are dried *in vacuo* and stored in the dark, also *in vacuo*, or in an exsiccator containing strong sulphuric acid. For use, the contents of one are dissolved in a little normal saline solution.

The comparison of the action of bacteria in the tissues in the production of these toxins to what takes place in the gastric digestion, has raised the question of the possibility of the elaboration by these bacteria of ferments, by which the process may be started. It would not be prudent to dogmatize as to whether the toxins do or do not belong to such an ill-defined group of substances as the ferments. It may be pointed out, however, that the essential concept of a ferment is that of a body which can originate change without itself being changed, and no evidence has been adduced that toxins fulfil this condition. Another property of ferments is that, so long as the products of fermentation are removed, the action of a given amount of ferment is indefinite. Again, in the case of toxins no evidence of such an occurrence has been found. A certain amount of a toxin is always associated with a given amount of disease effect, though a process of elimination of waste products must be all the time going on in the animal's body. Again, too much importance must not be attached to loss of toxicity by toxins at relatively low temperatures. Many proteids show a tendency to change at such temperatures; for instance, if egg albumen be kept long enough

at 55° C. nearly the whole of it will be coagulated. Such considerations suggest that the relation of toxic action to fermentation must be left an open question.

SIMILAR VEGETABLE AND ANIMAL POISONS.—Within recent years it has been found that the bacterial poisons belong to a group of toxic bodies all presenting very similar properties; other members of which occur widely in the vegetable and animal kingdoms. Among plants the best-known examples are the ricin and abrin poisons, obtained by making water emulsions of the seeds of the Riçinus communis and the Abrus precatorius (jequirity), respectively. The chemical reactions of ricin and abrin correspond to those of the bacterial toxins. They are soluble in water; they are precipitable in alcohol, but being less easily dialyzable than the albumoses they have been called toxalbumins. Their toxicity is seriously impaired by boiling, and they also gradually become less toxic on being kept. Both are among the most powerful poisons known, ricin being the more fatal.

It is also certain that the poisons of scorpions and of poisonous snakes belong to the same group. The poisons derived from the latter are usually called venins, and a very representative group of such venins derived from different species has been studied. To speak generally, there is derivable from the natural secretions of the poison glands a series of venins which have all the reactions of the bodies previously considered. Like ricin and abrin, they are not so easily dialyzable as bacterial toxins, and therefore they have also been classed as toxalbumins. While up to the present we have not been able to discover the exact chemical composition of any toxin, or even to obtain it in a pure state, many interesting facts upon the nature of toxins have been discovered by physiological methods.

Ehrlich's Theories as to the Nature of Extracellular Toxins.—From a large number of most carefully conducted experiments with the toxin and antitoxin of diphtheria, Ehrlich has formulated a theory concerning the constitution of the former. This theory has undergone several modifications since it was first proposed, and it is difficult to give an exact statement of it as it now stands. However, we will attempt to state in condensed form its essential points as follows:

Toxins and antitoxins neutralize one another after the manner of chemical reagents. The chief reasons for this belief lie in the observed facts: (*a*) that neutralization takes place more rapidly in concentrated than in dilute solutions, and (*b*) that warmth hastens and cold retards neutralization. From these observations Ehrlich concludes that toxins and antitoxins act as chemical reagents do in the formation of double salts. A molecule of the poison requires an exact and constant quantity of the antitoxin in order to produce a neutral or harmless substance. This implies that a specific atomic group in the toxin molecule combines with a certain atomic group in the antitoxin molecule.

The toxins, however, are not simple bodies, but easily split into other substances which differ from one another in the avidity with which they combine with antitoxin.

These derivatives Ehrlich calls prototoxins, deuterotoxins, and tritotoxins.

All forms of toxins are supposed to consist of two modifications, which combine in an equally energetic manner with antitoxin or with suitable receptors in the cells, but differ in their resistance to heat and other destructive agents.

The less-resistant form passes readily into a toxoid substance which has the same affinity for the antitoxin or the cell receptors as the original toxin, but is not poisonous. The facts observed, Ehrlich thinks, are best explained on the supposition that the toxic molecule contains two independent groups of atoms, one of which may be designated as the haptophorous and the other as the toxophorous group. It is by the action of the haptophorous group that toxin unites with antitoxin or the sensitive cell molecule.

The toxophorous group is unstable, but after its destruction the molecule still unites with the antitoxin or the sensitive molecule through its retained haptophorous group.

Specific antitoxins can be produced not only with toxins, but with toxoids.

Bordet believes, in contradistinction to Ehrlich, that toxin unites in different multiples with antitoxin, so that the toxin molecule may have its affinity slightly, partly, or wholly satisfied by antitoxin. Slightly satisfied, it is still feebly toxic; combined with a larger amount of antitoxin, it is not toxic; but still may, when absorbed into the system, lead to the production of antitoxin. Fully saturated, it has no poisonous properties and no ability to stimulate the production of antitoxin.

The most important of the extracellular toxins are those produced by the diphtheria and tetanus bacilli. These are very powerful; 0.0000001 gram of the dried filtrate of a tetanus culture will frequently kill a white mouse, while one-tenth of that amount of dried diphtheria filtrate has killed a guinea-pig.

The same bacterium may produce several entirely distinct toxins, thus, according to Madsen and Ehrlich, the specific tetanus poison consists of two toxins, tetanospasmin and tetanolysin. To the first of these the tetanic convulsions are due, while the second has a hæmolytic action.

When the tetanus toxins are placed in the blood tetanolysin largely combines with the blood corpuscles, while the tetanospasmin combines with the nerve cells. Each of these substances produces in animals a specific antitoxin. To obtain diphtheria and tetanus toxins for injection in animals the bacilli are grown in slightly alkaline beef-broth for from seven to ten days. The broth is then filtered and preserved. Tetanus toxin is produced under anaërobic conditions; diphtheria, under free access of oxygen. (See special chapters on these bacteria.)

Bacterial Endotoxins or Proteids.—The bacterial poisons which reside in the bodies of the bacteria are mostly yielded up only after the death of the organisms. Here, in the invaded animal, the disease effects are more closely associated with the actual presence of the bacteria

in the vicinity than in the case of the extracellular toxins. These substances are extracted by the method proposed by Koch and Buchner, of first crushing the bacteria in a moist or dried condition, and then of obtaining their contents with the aid partly of the hydraulic press. In this way a large series of impure bacterial proteids was obtained, which, though differing in some respects, exhibited mainly the same properties.

Altogether different from these poison effects are the immunization processes produced by the cell substances of bacteria, whether they be obtained from bacterial bodies or from chemical preparations. These processes have nothing to do with the toxic action of the cell proteids, but rather depend upon the introduction of suitable receptors which give rise to the bactericidal protective powers—lysin, precipitin, and agglutinin.

For the present we may assume with certainty that such receptors exist only in the unchanged bacterial cells, which, like cholera vibrio, pneumococcus, etc., give up *in toto* their destructive processes; on the other hand, we may say pretty surely that forcible extraction—that is, production of chemical proteid preparation—so changes most of the atomical grouping that little or no bactericidal reaction results from their introduction, and that these albuminous substances produce only the same reaction as other outside albuminous substances—*i. e.*, the formation of a specific precipitin, which, however, is closely allied to agglutinin. It is very probable, on the contrary, that in the substance so carefully prepared as Koch's tuberculin and Buchner's plasmin, from the tubercle bacillus and the cholera vibrio, the specific receptors may be retained, so that these preparations produce bactericidal and immunization processes.

SUMMARY.—1. One group of bacteria produces as free secretions true toxins. After extraction of this soluble poison there remains a pure unspecific bacterial residue. Type: diphtheria.

2. Another large group possesses apparently only endotoxins, true toxins which are more or less closely bound to the living cell, and which are only in a small degree separable in unchanged condition perhaps outside of the body. On death of the cell they become partly free, partly remain united, or become secondary poisonous modifications no longer of the nature of toxins. In this group, therefore, the dead cell bodies cannot be entirely freed without residue from the poisons; the pure proteid cannot be clearly identified by its individual action. With this reservation, however, the proteid action can be demonstrated. Type: cholera, typhoid, pneumococcus.

3. A third group yields perhaps no true toxins, not even intraplasmatically. The cell plasma contains poisons of another kind which obscures the typical proteid action. Type: anthrax, tuberculosis. Possibly by further investigation Groups 2 and 3 may be united.

The pyogenic action of their proteids is common to all bacteria, this depending principally upon their being extraneous albuminous substances. Pyogenic effects may be produced in like manner by

extraneous albumins of non-bacterial origin. That every extraneous albuminous substance is harmful to the organism which seeks to resist its action is shown by those specific precipitating ferments, the precipitins, which are produced in the organisms after the introduction of every extraneous albumin.

Sulphuretted Hydrogen.—Sulphuretted hydrogen is a very common bacterial product. Its presence is determined by pasting a piece of paper moistened with lead acetate inside the neck of the flask containing the culture, closing the mouth with a cotton-wool stopper, and over this again an India-rubber cap (black rubber free from sulphur). The paper is colored at first brownish and later black; repeated observation is necessary, as the color sometimes disappears toward the end of the reaction. Apparently negative results should not be rashly accepted as conclusive.

Sulphuretted hydrogen may be formed:

1. From albuminous substances. This power, according to Petri and Maassen, of forming sulphuretted hydrogen, particularly in liquid culture media containing much peptone (5 to 10 per cent.) is possessed, though in different degree, by all bacteria examined by them; only a few bacteria form H_2S in bouillon in the absence of peptone, while about 50 per cent. in media containing 1 per cent. peptone.

2. From powdered sulphur. All bacteria produce in culture media to which pure powdered sulphur is added considerably more H_2S than without this addition. Petri and Maassen suggest that this is due to the nascent hydrogen produced by the bacteria.

3. From thiosulphates and sulphites. Studied particularly in yeast, but demonstrated also by Petri and Maassen in several bacteria.

The presence of sugar in the culture does not affect the production of H_2S by bacteria, but saltpetre reduces it, nitrites being formed. The absence of oxygen favors the production of H_2S. Light diminishes the development of H_2S by facultative anaërobes, sulphates being formed instead.

Reduction Processes.—All bacteria, as we have seen, possess the property of converting sulphur into sulphuretted hydrogen, for which purpose is required the presence of nascent hydrogen. The following processes depend also in part upon the action of nascent hydrogen:

1. The reduction of blue litmus pigments, methylene blue, and indigo to colorless substances. The superficial layer of cultures in contact with the air shows often no reduction, only the deeper layers being affected. By agitation with access of air the colors may be again restored, but, at the same time, if acid has been formed, the litmus pigment is turned red. According to Cohn, the property of reducing litmus belongs to all liquefying bacteria, but some non-liquefying species also possess it.

2. The reduction of nitrates to nitrites and ammonia. The first of these properties seems to pertain to a great many bacteria—at least Petri and Maassen found in six species, grown in bouillon containing 2.5 to 5 per cent. peptone and 0.5 per cent. nitrate, that almost all pro-

duced nitrite abundantly; once only was ammonia observed. In a number of bacteria studied by Rubner only one failed to produce nitrite. The test for nitrites is made as follows: Two bouillon tubes containing nitrates are inoculated, and, along with two uninoculated tubes, are allowed to remain in the incubator for several days; then to the cultures and control test is added a small quantity of colorless iodide of starch solution (thin starch paste containing 0.5 per cent. potassium iodide) and a few drops of pure sulphuric acid. The control tubes remain colorless or become gradually slightly blue, while if nitrites are present a dark-blue or brown-red coloration is produced.

The demonstration of ammonia is made by the addition of Nessler's reagent to culture media free from sugar. In bouillon, if ammonia be present, Nessler's reagent is almost immediately reduced to black mercurous oxide. A strip of paper saturated with the reagent can also be suspended over the bouillon tube, or this can be distilled at a low temperature with the addition of magnesium oxide and the distillate treated with Nessler's reagent. A yellow to red coloration indicates the presence of ammonia. Controls are necessary.

Aromatic Products of Decomposition.—Many bacteria produce aromatic substances as the result of their growth. The best known of these are indol, skatol, phenol, and tyrosin. Systematic investigations have only been made with regard to the occurrence of indol and phenol.

TEST FOR INDOL.—To a bouillon culture, which should, if possible, be not under eight days old and free from sugar, is added half its volume of 10 per cent. sulphuric acid. If in heating to about 80° C. a pink or bluish-pink coloration is immediately produced it indicates the presence of both indol and nitrites, the above-described nitroso-indol reaction requiring the presence of both of these substances for its successful operation. This is the so-called "cholera-red reaction," but it may be applied to many other spirilla besides cholera. As a rule, however, the addition of sulphuric acid alone is not sufficient, and a little nitrite must be added; this may be done later, the culture being first warmed without nitrite, when, if there is no reaction or a doubtful one, 1 to 2 c.c. of 0.005 per cent. solution of sodium nitrite is added until the maximum reaction is obtained. The addition of strong solutions of nitrite colors the acid liquid brownish-yellow and ruins the test. Out of sixty species examined by Lehmann, twenty-three gave the indol reaction.

Decomposition of Fats.—Pure melted butter is not a suitable culture medium for bacteria. The rancidity of butter is brought about (1) as the result of a purely chemical decomposition of the butter by the oxygen of the air under the influence of sunlight, and (2) through the formation of lactic acid from the milk-sugar left in the butter. Fats are, however, attacked by bacteria when mixed with gelatin and used as culture media, with the consequent production of acid.

Putrefaction.—By putrefaction is understood in common parlance every kind of decomposition due to bacteria which results in the production of malodorous substances. Scientifically considered, putrefaction depends upon the decomposition of complex organic compounds,

albuminous substances, which are frequently first peptonized and then further decomposed. Typical putrefaction occurs only when oxygen is absent or scanty; the free passage of air through a culture of putrefactive bacteria—an event which does not take place in natural putrefaction —very much modifies the process: first, biologically, as the anaërobic bacteria are inhibited, and then by the action of the oxygen on the products or by-products of the aërobic and facultative anaërobic bacteria.

As putrefactive products we have peptone, ammonia, and amines, leucin, tyrosin, and other amido substances; oxyfatty acids, indol, skatol, phenol, ptomains, toxins, and, finally, sulphuretted hydrogen, mercaptans, carbonic acid, hydrogen, and, possibly, marsh-gas.

Nitrifying Bacteria.—According to recent observations, nitrification is produced by a small, special group of bacteria, cultivated with difficulty, which do not grow on our usual culture media. From the investigations of Winogradsky it would appear that there are two common micro-organisms present in the soil, one of which converts ammonia into nitrites and the other converts nitrites into nitrates.

Conversion of Nitrous and Nitric Acids into Free Nitrogen.—This process is performed by a number of bacteria. The special nitrate-fermenting bacteria, however, were first accurately described by Barri and Stutzer. In their exhaustive investigation they first isolated from horse-manure two bacteria, neither of which was alone capable of producing nitrogen from nitrates, but which together in the presence of oxygen, but never without it entirely, decomposed nitrates energetically. Later a second denitrificating bacillus was found, B. denitrificans II, which by itself was able to produce nitrogen from nitrates.

The practical importance of these organisms is that by their action large quantities of nitrates in the soil, and especially in manure, may become lost as plant food by being converted into nitrogen.

Nitrogen Combination.—The *bacillus radiocola* of Beyerinck, which was isolated by him, has the power of assimilating nitrogen from the air. This bacillus is found in the small root nodules of various leguminous plants (pease, clover, etc.), and can be obtained from these in cultures. Different varieties exist in different kinds of legumes, each kind of legume apparently having a special variety of bacteria adapted to it, and not every variety is capable of producing nodules in all legumes. There are certain "neutral" varieties, however, existing free in the soil and not adapted to any special legume, and these seem to be able to form nodules in different legumes.

By the aid of these root bacteria, which gain entrance to the roots and there produce this nodular formation, the leguminous plants are enabled to assimilate nitrogen from the atmosphere. This explains the reason why poor, sandy soils become gradually fruitful when pease, lupine, and other varieties of legumes are grown upon them and then turned under with the plough. It is not known exactly how this assimilation of nitrogen occurs, but it is assumed that the zoöglœa-like bacteria, called *bacteroids*, constantly observed in the nodules, either alone

or in a special degree, possess the property of assimilating and combining nitrogen. It seems, moreover, to have been recently established that, independently of the assistance of the legumes, certain nodule bacteria exist free in the soil, which accumulate nitrogen by absorbing it from the air (Stutzer).

Formation of Acids from Carbohydrates.—Free acids are formed by many bacteria in culture media containing sugar; the production of acid in ordinary bouillon takes place on account of the presence of grape-sugar, which is usually derived in small quantities from the meat.[1] According to Theobald Smith, all anaërobic or facultative anaërobic bacteria form acids from sugar; the strict aërobic species do not, or so very slowly that the acid is concealed by the almost simultaneous production of alkali. The formation of acid occurs sometimes with and sometimes without the production of gas. Excessive acid production may cause the death of the bacteria from the increase in acidity of the culture media.

If after the sugar is consumed not enough acid has been formed to kill the bacteria, the acid is neutralized gradually and in the end the reaction becomes alkaline.

Among the acids produced the most important is lactic acid; also traces of formic acid, acetic acid, proprionic acid, and butyric acid, and not infrequently some ethyl-alcohol and aldehyde or acetone are formed. Occasionally no lactic acid is present, and only the other acids are formed.

Various bacteria, as yet incompletely studied, possess the property of producing butyric acid and butyl-alcohol from carbohydrates.

Some bacteria also seem to have the power of decomposing cellulose.

Formation of Gas from Carbohydrates and Other Fermentable Substances of the Fatty Series.—The only gas produced in *visible* quantity in sugar-free culture media is nitrogen. If sugar is vigorously decomposed by bacteria, as long as pure lactic acid or acetic acid is produced there may be no development of gas, as, for instance, with the B. typhosus on grape-sugar; but frequently there is much gas developed, especially in the absence of air. About one-third of the acid-producing species also develop gas abundantly, this consisting chiefly of CO_2, which, according to Smith, is always mixed with H. Marsh-gas is seldom formed by bacteria, with the exception of those decomposing cellulose.

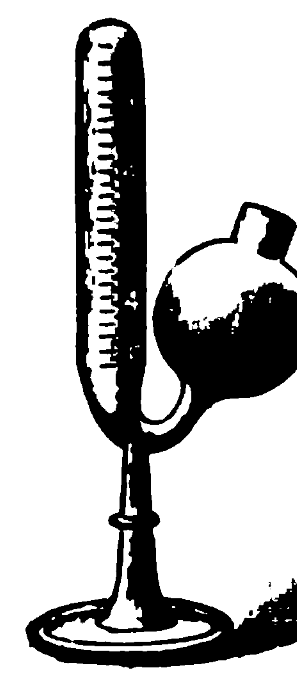
In order to test the production of gas, a culture medium composed of glucose-agar, containing about 1 per cent. grape-sugar, may be used. At the end of eight to twelve hours in the incubator (or twenty-four hours' room temperature) the agar will be seen to be full of gas-bubbles or broken up into holes and fissures.

For the determination of the quantity and kind of gas produced by a given micro-organism the fermentation tube recommended by Theobald Smith is the best. This is a bent tube, constricted greatly at its lowest

[1] According to Theobald Smith, 75 per cent. of the beef ordinarily bought in the markets contains appreciable quantities of sugar (up to 0.3 per cent.).

portion, supported upon a glass base, as shown in Fig. 64. The tube is filled with a culture media consisting of 1 per cent. glucose, peptone bouillon (without air bubbles), and sterilized in the steam sterilizer. It is then inoculated with a loopful of a culture of the organism in question, and observations taken:

FIG. 64



Fermentation tube.

1. If there is a turbidity produced in the open bulb it indicates the presence of an aërobic species; if this clouding occurs only in the closed arm, while the open bulb remains clear, it is an anaërobic species.

2. The quantity of gas produced daily should be marked on the upright arm; if the tube is graduated a note of it is taken and the percentage calculated on the fourth to the sixth day after gas production has ceased.

3. A rough analysis of the gas produced may be made as follows: Having signified by a mark on the tube the quantity of gas produced, the open bulb is completely filled with a 10 per cent. solution of soda, the mouth tightly closed with the thumb, and the mixture thoroughly shaken. After a minute or two all the gas is allowed to rise to the top of the closed arm by inclining and turning the tube, and then, removing the thumb, the volume of gas left after the union of the NaOH with the CO_2 is noted. The remainder is nitrogen, hydrogen, and marsh-gas. If it is desired to test for the presence of hydrogen, the thumb is again placed over the open end and the gas collected under it. As the thumb is moved a lighted match is brought in contact with the gas. If hydrogen is present a slight explosion occurs.

Formation of Acids from Alcohol and Other Organic Acids.—It has long been known that the bacterium aceti and other allied bacteria convert dilute solutions of ethyl-alcohol, under the influence of oxidation, into acetic acid:

$$\begin{array}{c} CH_3 \\ | \\ CH_2OH \end{array} + O_2 + \begin{array}{c} CH_3 \\ | \\ COOH. \end{array} + H_2O.$$

The higher alcohols—glycerin, dulcit, mannite, etc.—are also converted into acids—glycerin, indeed, as commonly as sugar.

Finally, numerous results have been obtained from the conversion of the fatty acids and their salts into other fatty acids by bacteria. As a rule, the lime-salts of lactic, malic, tartaric, and citric acids have been employed, these being converted into various acids by the action of bacteria, such as butyric, proprionic, valerianic, and acetic acids; also succinic acid, ethyl-alcohol, and, more rarely, formic acid have been produced. Among the gases formed were chiefly CO_2 and H.

Thus Pasteur found that anaërobic bacteria convert lactate of lime into butyric acid.

CHAPTER VIII.

THE EFFECT OF VARIOUS DELETERIOUS INFLUENCES UPON BACTERIA.

Influence of Electricity on Bacteria.—The majority of the observations heretofore made on this subject would seem to indicate that there is no direct action of the galvanic current on bacteria; but the effect of heat and the electrolytic influence on the culture liquid may produce changes which finally sterilize it.

Influence of Agitation.—Meltzer has shown that the vitality of bacteria is destroyed by protracted and violent shaking, which causes a disintegration of the cells. Appel found that moderate agitation of the bacteria caused no injury, even when long continued.

Influence of Pressure.—D'Arsonval and Charrin submitted a culture of bacillus pyocyaneus to a pressure of fifty atmospheres under carbonic acid. At the end of four hours cultures could still be obtained, but the bacillus had lost its power of pigment production. A few colonies were developed after six hours' exposure to this pressure, but after twenty-four hours no development occurred.

Influence of Light.—A large number—perhaps the majority—of bacteria are inhibited in growth by the action of bright daylight, all are by that of direct sunlight, and when the action of the latter is prolonged they lose their power of developing when later placed in the dark.

In order to test the susceptibility of bacteria to light, it is best, according to H. Buchner, to suspend a large number of bacteria in nutrient gelatin or agar and pour the media while still fluid in Petri dishes, upon which has been pasted a strip of black paper on the side upon which the light is to act. The action of heat may be shut off by allowing the ray of light to first pass through a layer of water or alum of several centimetres' thickness. After the plates have been exposed to the light for one-half, one, one and a half, two hours, etc., they are taken into a dark room and allowed to stand at 20° or 35° C. a sufficient length of time to allow of growth, and then examined to see whether there are colonies anywhere except on the spot covered by the paper; when the colonies exposed to the light have been completely destroyed there is a sharply defined region of the shape of the paper strip crowded with colonies lying in a clear sterile field.

Dieudonné, in experiments upon the bacillus prodigiosus, found that direct sunlight in March, July, and August killed these bacilli in one and a half hours; in November in two and a half hours. Diffuse daylight in March and July restrained development after three and a half hours' exposure (in November four and a half hours), and com-

pletely destroyed vitality in from five to six hours. Electric arc-light inhibited growth in five hours and destroyed vitality in eight hours. Incandescent light inhibited growth in from seven to eight hours and killed in eleven hours. Similar results have been obtained with B. coli, B. typhosus, and B. anthracis. According to Koch, the tubercle bacillus is killed by the action of direct sunlight in a time varying from a few minutes to several hours, depending upon the thickness of the layer exposed and the season of the year. Diffuse daylight also had the same effect, although a considerably longer time of exposure was required—when placed close to a window, from five to seven days.

Only the ultraviolet, violet, and blue rays of the spectrum seem to possess bactericidal action; green light has very much less so; red and yellow light not at all. The action of light is apparently assisted by the admission of air; anaërobic species, like the tetanus bacillus, and facultative anaërobic species, such as the colon bacillus, are able to withstand quite well the action of sunlight in the absence of oxygen, the B. coli intense direct sunlight for four hours.

According to Richardson and Dieudonné, the mechanism of the action of light may be at least partially explained by the fact that in agar plates exposed to light for a short time (even after ten minutes' exposure to direct sunlight) hydrogen peroxide (H_2O_2) is formed. This is demonstrated by exposing an agar plate half covered with black paper, upon which a weak solution of iodide of starch is poured, and over this again a dilute solution of sulphate of iron; the side exposed to the light turns blue-black. In gases containing no oxygen, hydrogen peroxide is not produced, and the light has no injurious effect. Access of oxygen also explains the effect which light produces on culture media which have been exposed to the action of sunlight, as standing in the sun for a time, when afterward used for inoculation. The bacteria subsequently introduced into such media grow badly—far worse than in fresh culture media which are kept in the shade.

Influence of Radium.—*Radioactive fluids* have a slight inhibiting effect on bacterial growth, but nothing decided enough to be used for therapeutic purposes.

Influence of X-rays.—These rays have a slight inhibiting effect on bacteria when they are directly exposed to them.

Influence of One Species upon the Growth of Another.—While it is the custom of bacteriologists to have pure cultures to work with, we should never forget that in nature bacteria often occur in mixed cultures. If we examine water, milk, or the contents of the intestines of either sick or healthy persons we shall always find several species of bacteria occurring together. This admixture may, perhaps, seem to us at first merely accidental, but on further investigation it will appear also that in the department of bacteriology there exist synergists and antagonists, or at least bacteria which assist or oppose one another mutually or one-sidedly. Nencki speaks of *symbiosis* and *enantobiosis*.

The existence of antagonisms can be demonstrated experimentally by inoculating gelatin streak cultures of various bacteria. It is found

that many species will not grow at all or only sparingly when in close proximity to some other species. This antagonism, however, is often only one-sided in character; for instance, the bacillus fluorescens putidus grows well when inoculated between streaks of staphylococcus, but the latter micrococcus will not grow at all when inoculated between cultures of the bacillus putidus, the growth of the staphylococcus remaining scanty when the two species are inoculated simultaneously. Again, when gelatin or agar plates are made from two different species of bacteria it may be observed that only one of the two grows. A third method of making this experiment is to simultaneously inoculate the same liquid medium with two species, and then examine them later, both microscopically and by making plate cultures; not infrequently the one species may take precedence of the other, which it finally overcomes entirely. The practical application of this is to make sufficient dilutions of material when plating for the estimation of the number of bacteria or the isolation of pure cultures.

Finally, bacteria may oppose one another as antagonists in the animal body. As Emmerich has shown, animals infected with anthrax may often be cured by a secondary infection with the streptococcus.

The symbiotic or co-operative action of bacteria is of still greater importance, of which the following examples may be given:

1. Some bacteria thrive better in association with other species than alone. Brueger has recently shown that pneumococci when grown together with a bacillus obtained from the throat, produce very large, succulent colonies. Certain anaërobic species grow even with the admission of air if only other aërobic species are present (tetanus).

2. Certain chemical effects, as, for instance, the decomposition of nitrates to gaseous nitrogen, cannot be produced by many bacteria alone, but only when two are associated.

3. Attenuated varieties of bacteria may regain virulence when grown in contact with other bacteria.

Duration of Life in Pure Water.—When bacteria which require much organic food for their development, and these include most of the pathogenic species, are placed in distilled water they soon die—that is, within a few days; even in sterilized well water or surface water their life duration does not usually exceed eight to fourteen days, and they rarely multiply. Instances, however, of much more extended life under certain conditions are recorded.

Effect of Drying.—Want of water affects bacteria in different ways. Upon dried culture media development soon ceases; but in media dried gradually at the room temperature (nutrient agar, gelatin, potato) they live often for a long time, even when there are no spores to account for it. A shrunken residue of such cultures in bouillon has often been found, after a year or more, to yield living bacteria. The question as to how long the non-spore bearing forms are capable of retaining their vitality when dried on a cover-glass or silk threads has been variously answered. We know now that there are many factors which influence the retention of vitality. The following table of the results obtained

by Sirena and Alessi gives some idea of the extent and effect of such influences. In the experiments silk threads were saturated with bouillon cultures or aqueous suspensions of the bacteria, and some then enclosed in tubes containing sulphuric acid or calcium chloride, while others were left exposed to various outside influences:

Desiccation.	With sulphuric acid, killed at end of	With calcium chloride, killed at end of	In incubator at 37°, killed at end of	In dry room in shade, killed at end of	In moist room, killed at end of
Cholera spirilla . .	1 day	1 day	1 day	1 day	12 days
B. of fowl cholera .	2 days	1 "	1 "	5 days	59 "
B. typhosus. . . .	41 "	1 "	18 days	64 "	68 "
B. mallei	35 "	44 days	31 "		
Diplococ. pneumoniæ	114 "	31 "	131 "	164 "	192 "

The results of all investigators, however, would seem to indicate that the greatest possible care must be exercised in desiccation experiments to come to any positive conclusions; but recently most astonishing results have been obtained with regard to many species usually supposed to be particularly sensitive to desiccation, showing that under certain conditions they may retain their vitality in a dry state for a very long time. Thus, Koch found that cholera spirilla lived only a few hours when dry; Kitasato determined their life duration at fourteen days at most; while various French observers have found that they may, under favorable conditions, live 150 to 200 days. The varying results sometimes reported by different observers in such experiments may be explained by the fact that the conditions under which they were made were different, depending upon the desiccator used, the medium upon which the cultures were grown, and the use of silk threads or cover-glasses. In all these experiments, of course, it should be previously determined that in spore-bearing species there are no spores present. Even when a dried culture lives for a long time the majority of the organisms die in a few hours after drying. We have found 1,500,000 colon bacilli to be reduced to 100,000 after three hours' drying. When protected by a covering of mucus, as in expectoration, they live much longer than when unprotected.

Behavior toward Oxygen and Other Gases.—As already noted under the nutritious substances required by bacteria, it is customary to divide bacteria into three classes, according to their behavior toward oxygen.

1. Aërobic Bacteria.—Growth only in the presence of oxygen: the slightest restriction of air inhibits development. Spore formation especially requires the free admission of air.

2. Anaërobic Bacteria.—Growth and spore formation only in the total exclusion of oxygen. Among this class of bacteria are the bacillus of malignant œdema, the tetanus bacillus, the bacillus of symptomatic anthrax, and many soil bacteria. Exposed to the action of oxygen, the vegetative forms of these bacteria are readily destroyed; the spores,

on the contrary, are very resistant. Anaërobic bacteria being deprived of oxygen—the chief source of energy supplied to the aërobic species, by which they oxidize the nutritive substances in the culture media—they are dependent for their nutrition upon decomposable substances, such as grape-sugar, which on separating into two smaller molecules, alcohol and carbonic acid, give out energy or heat. Anaërobic bacteria, therefore, require for their cultivation, as a rule, media containing glucose or some equivalent.

3. Facultative Aërobic and Facultative Anaërobic Bacteria. —The greater number of aërobic bacteria, including most of the pathogenic species, are capable of withstanding, without being seriously affected, some restriction in the amount of oxygen admitted, and many, indeed, grow equally luxuriantly in the partial exclusion of oxygen. Life in the animal body, for example, as in the intestines, necessitates existence with diminished supply of oxygen. Pigment formation almost always ceases with the exclusion of oxygen, but poisonous products of decomposition may be more abundantly produced.

It is important to note that, according to recent investigations, it has been shown that the aërobic development of the anaërobes may be facilitated by the presence of living or dead aërobes.

It has also been observed not infrequently that certain species which on their isolation at first showed more or less anaërobic development —that is, a preference to grow in the depth of an agar stick culture, for instance—after a while seem to become strict aërobes, growing only on the surface of the medium. This observation proves that the simple fact of an organism showing aërobic instead of anaërobic growth is not sufficient for its separation into a distinct species.

While all facultative as well as strict anaërobes grow well in nitrogen and hydrogen, they behave very differently toward carbonic acid gas. A large number of these species do not grow at all, being completely inhibited in their development until oxygen is again admitted—for example, B. anthracis and B. subtilis and other allied species. It has been found in some species, as glanders and cholera, that the majority of the organisms are quickly killed by CO_2, while a few offer a great resistance, rendering impossible complete sterilization by means of this gas. Another group, again—viz., streptococcus and staphylococcus—exhibits a scanty growth; while a third group, like the B. typhosus and B. prodigiosus, is not at all affected, growing equally as well in the presence of oxygen, and the liquefaction, even of gelatin, not being interfered with; only, on account of the lack of oxygen, there is no pigment formation. Finally, a mixture of one-fourth air to three-fourths carbonic acid gas seems to have no injurious effect on bacteria which cannot grow in an atmosphere of pure CO_2.

Sulphuretted hydrogen in large quantity is a strong bacterial poison, and even in small amounts kills some bacteria.

CHAPTER IX.

THE DESTRUCTION OF BACTERIA BY CHEMICALS—PRACTICAL USE OF DISINFECTANTS.

Many chemical substances, when brought in contact with bacteria, unite with their cell substance. New compounds are thus formed, and the life of the bacteria and the disinfecting properties of the substances are usually destroyed; while in the vegetative stage bacteria are much more easily killed than when in the spore form, and their life processes are inhibited by substances less deleterious than those required to destroy them.

Bacteria, both in the vegetative and in the spore form, differ among themselves considerably in their resistance to the poisonous effects of chemicals. The reason for this is not as yet clear, but is apparently connected with the structure and chemical nature of their cell substance.

Chemicals are more poisonous at fairly high than at a low temperature, and act more quickly upon bacteria when they are suspended in fluids singly than when in clumps, and in pure water rather than in solutions containing organic matter. The increased energy of disinfectants at higher temperatures indicates in itself a probability that a true chemical reaction takes place. In estimating the extent of the destructive action of chemicals the following degrees are usually distinguished:

1. The growth is not permanently interfered with, but the pathogenic and zymogenic functions of the organism are diminished—*attenuation.*
2. The organisms are not able to multiply, but they are not destroyed by antiseptic action.
3. The vegetative development of the organisms is destroyed, but not the spores—incomplete sterilization.
4. Vegetative and spore formation are destroyed. This is complete *sterilization* or *disinfection.*[1]

The methods employed for the determination of the germicidal action of chemical agents on bacteria are, briefly, as follows:

If it is desired to determine what is the minimum concentration of the chemical substance required to produce complete inhibition of growth we proceed thus: A 10 per cent. solution of the disinfectant is prepared and 1 c.c., 0.5 c.c., 0.3 c.c., 0.1 c.c., etc., of this is added to 10 c.c. of liquefied gelatin, agar, or bouillon, or, more accurately, 10 c.c.

[1] Disinfection strictly defined is the destruction of all organisms and their products which are capable of producing disease. Sterilization is the destruction of all saprophytic as well as parasitic bacteria. Practically, however, the two terms are used interchangeably as meaning the destruction of all living bacteria.

minus the amount of solution added, in so many tubes. The tubes then contain 1 per cent., 0.5 per cent., 0.3 per cent., and 0.1 per cent. of the disinfectant. The fluid media in the tubes are then inoculated with a platinum loopful of the test bacteria. The melted agar and gelatin may be simply shaken and allowed to remain in the tubes, and watched as to whether any growth takes place, or the contents of the tubes are poured out into Petri dishes, where the development or lack of development of colonies and the number can be observed. The same test can be made with material containing only spores.

If it is desired to determine the degree of concentration required for the destruction of vegetative development, the organism to be used is cultivated in bouillon, and to each of a series of tubes holding in watery solution different percentages of the disinfectant a few drops of the culture from which all lumps have been filtered are added. At intervals of one, five, ten, fifteen, and thirty minutes, one hour, and so on a small platinum loopful of the mixture is taken from each tube and inoculated into 10 c.c. of fluid agar or gelatin, from which plate cultures are made. The results obtained are signified as follows: x per cent. of the disinfectant in watery solution and at x temperature kills the organism in twenty minutes, y per cent. kills in one minute, and so on. If there be any doubt whether the trace of the disinfectant carried over with the platinum loops may have rendered the gelatin unsuitable for growth, thus falsifying results, control cultures should be made with vigorous bacteria in gelatin to which a similar trace of the disinfectant has been added. If the strength of the disinfectant is to be discovered in different substances it must be tested in these substances and not in water.

The disinfectant to be examined should always be dissolved in an inert fluid, such as water; if on account of its being difficultly soluble in water, it is necessary to use alcohol for its solution, control experiments may be required to determine the action of the alcohol on the organism. Sometimes, as in the case of corrosive sublimate, the chemical unites with the cell substance to form an unstable compound, which inhibits the growth of the organism only while the union exists. If this compound is not broken up in the media, it will probably not be in the body. In some tests it is of interest to break up this union and note then whether the organism is alive or dead.

In the above determinations the absolute strength of the disinfectant required is considerably less when culture media rich in albumin are employed than when the opposite is the case. Cholera spirilla grown in bouillon containing no peptone or only 0.5 per cent. of peptone are destroyed in half an hour by 0.1 per cent. of hydrochloric acid; grown in 2 per cent. peptone-bouillon their vitality is destroyed in the same time on the addition of 0.4 per cent. HCl. In any case the organisms to be tested should all be treated in exactly the same way and the results accompanied by a statement of the conditions under which the tests were made.

The following table gives the results and methods used in an actual experiment to test the effect of blood serum upon the disinfecting action of bichloride of mercury and carbolic acid upon bacteria:

TEST FOR THE DIFFERENCE OF EFFECT OF BICHLORIDE OF MERCURY AND CARBOLIC ACID SOLUTIONS ON ANTHRAX AND TYPHOID BACILLI IN SERUM AND BOUILLON.

Time . . .	1′	3′	5′	10′	20′	30′	45′	1 hr.	1½ hrs.	2 hrs.	
A. Serum . . . 2.5 c.c. $HgCl_2$ sol. 1 : 1000 2.5 c.c. Anthrax threads . . .	+	+	+	+	+	—	+	—	—	—	Solution equals 1 : 2000 bichloride.
B. Bouillon . . 2.5 c.c. $HgCl_2$ sol. 1 : 1000 2.5 c.c. Anthrax threads . . .	—	—	—	—	—	—	—	—	—	—	Same.
C. Serum . . . 2.5 c.c. Carbolic sol. 5 % 2.5 c.c. Typhoid threads . . .	+	+	+	+	+	+	+	—	—	—	Solution equals 2½ % carbolic acid.
D. Bouillon . . 2.5 c.c. Carbolic sol. 5 % 2.5 c.c. Typhoid threads . . .	+	+	+	+	+	+	—	—	—	—	Same.

— Indicates total destruction of bacteria with no growth in media.
+ Indicates lack of destruction of bacteria with growth in media.

Pieces of sterile thread (one inch) were placed in bouillon cultures of anthrax and typhoid bacilli for ten minutes, then removed to Petri dishes, and dried in the incubator for twenty-four hours. These were then placed in serum and bouillon respectively (2.5 c.c.). From each a control was taken. Then 2.5 c.c. $HgCl_2$ (1 :1000) and carbolic solution (5 per cent.) was added to either, as shown in A, B, C, and D. From each one thread was taken at varying periods of time and planted in bouillon tubes. The threads from A and B ($HgCl_2$ solution) were washed in sterile water, then in a solution of ammonium sulphide (25 per cent.) to remove the $HgCl_2$, then in sterile water again, then in the bouillon. The threads from the carbolic solution were washed in sterile water before planting.

Observations: The serum seems to have an inhibitory action with the bichloride solution, allowing a growth up to forty-five minutes, while with the bouillon the action is much quicker, preventing a growth after an exposure of one minute or over. With the carbolic acid solution the serum made less difference in the results.

Many substances which are strong disinfectants become altered under the conditions in which they are used, so that they lose a portion or all of their germicidal properties; thus, quicklime and milk of lime act by means of their alkali and are disinfecting agents only so long as sufficient calcium hydroxide is present. If this is changed by the carbon dioxide of the air into carbonate of lime it becomes harmless. Bichloride of mercury and many other chemicals form compounds with many organic and inorganic substances, which, though still germicidal, are much less so than the original substances.

The Disinfecting Properties of the More Commonly Used Disinfectants.

Bichloride of Mercury.—This substance, when present in 1 part in 1,000,000 nutrient gelatin or bouillon, prevents the development of parasitic bacteria. In water 1 part in 500,000 will kill many varieties in a few minutes, but in bouillon twenty-four hours may be needed. With organic substances its power is lessened, so that 1 part to 1000 may be required. Most spores are killed in 1:1000 watery solution within one hour. Corrosive sublimate, as already noted, is less effective as a germicide in alkaline fluids containing much albuminous substance than in watery solution. In such fluids, besides loss in other ways, precipitates of albuminate of mercury are formed which are at first insoluble, so that a part of the mercuric salt does not really exert any action. In alkaline solutions, such as blood, blood serum, pus, tissue fluids, etc., the soluble compounds of mercury are converted into oxides or hydroxides. The soluble compounds can, of course, remain in solution only when there are present sufficient quantities of certain bodies which render solution possible. Bodies of this sort are especially the alkaline chlorides and iodides, and, above all, sodium chloride and ammonium chloride. A very simple way of preventing precipitation of the mercury, then, is to add a suitable quantity of common salt to the corrosive sublimate. Those compounds of mercury which, like the cyanides, are not precipitated with alkalies, because they at once form double salts, require no addition of salt.

For ordinary use, where corrosive sublimate is employed, solutions of 1:500 and 1:1000 will suffice, when brought in contact with bacteria in that strength, to kill the vegetative forms within fifteen minutes, the stronger solution to be used when much organic matter is present.

Biniodide of Mercury.—This salt is very similar in its effects to the bichloride. It is somewhat more powerful.

Nitrate of Silver.—Nitrate of silver in solution has about one-fourth the value of the bichloride of mercury as a disinfectant, but nearly the same value in inhibiting growth.

Sulphate of Copper.—This salt has about 50 per cent. of the value of mercuric chloride. It has a quite remarkable affinity for many forms of algæ, so that when in water 1:1,000,000 it destroys many forms; 1:400,000 destroys typhoid bacilli in twenty-four hours when the water has no excessive amount of organic material. It is not known to be poisonous in this strength, so that it can be temporarily added to water supplies.

Sulphate of Iron.—This is a much less powerful disinfectant than sulphate of copper.

Sodium Compounds.—A 30 per cent. solution of NaOH kills anthrax spores in about ten minutes, and in 4 per cent. in about forty-five minutes. Sodium carbonate kills spores with difficulty even in concentrated solution, but at 85° C. it kills spores in from eight to ten minutes. A 5 per cent. solution kills in a short time the vegetative forms of bacteria.

Even ordinary soapsuds have a slight bactericidal as well as a marked cleansing effect. The bicarbonate has almost no destructive effect on bacteria.

Calcium Compounds.—Calcium hydroxide $Ca(OH)_2$ is a powerful disinfectant; the carbonate, on the other hand, is almost of no effect. A 1 per cent. watery solution of the hydroxide kills bacteria which are not in the spore form within a few hours. A 3 per cent. solution kills typhoid bacilli in one hour. A 20 per cent. solution added to equal parts of feces or other filth and mixed with them will completely sterilize them within one hour.

Effect of Acids.—An amount of acid which equals 40 c.c. of normal hydrochloric acid per litre is sufficient to prevent the growth of all varieties of bacteria and to kill many. Twice this amount destroys most bacteria within a short time. The variety of acid makes little difference. Bulk for bulk, the mineral acids are more germicidal than the vegetable acids, but that is because their molecular weight is so much less. A 1:500 solution of sulphuric acid kills typhoid bacilli within one hour. Hydrochloric acid is about one-third weaker, and acetic acid somewhat weaker still. Citric, tartaric, malic, formic, and salicylic acids are similar to acetic acid. Boric acid destroys the less resistant bacteria in 2 per cent. solution and inhibits the others.

Gaseous Disinfectants.

The germicidal action of gases is much more active in the presence of moisture than in a dry condition.

Numerous experiments have been made with sulphur **dioxide** gas (SO_2), owing to the fact that it has been so extensively used for the disinfection of hospitals, ships, apartments, clothing, etc. This gas is a much more active germicide in a moist than in a dry condition; due, no doubt, to the formation of the more active disinfecting agent —sulphurous acid (H_2SO_3). In a pure state anhydrous sulphur dioxide does not destroy spores, and is not certain to destroy bacteria not in spore form. Sternberg has shown that the spores of the bacillus anthracis and bacillus subtilis are not killed by contact for some time with liquid SO_2 (liquefied by pressure). Koch found that various species of spore-bearing bacilli exposed for ninety-six hours in a disinfecting chamber to the action of SO_2, in the proportion of from 4 to 6 per cent. by volume, were not destroyed. In the absence of spores, however, the anthrax bacillus in a moist condition, attached to silk threads, was found by Sternberg to be destroyed in thirty minutes in an atmosphere containing 1 volume per cent. As the result of a large number of experiments with SO_2 as a disinfectant it has been determined that an "exposure for eight hours to an atmosphere containing at least 4 volumes per cent. of this gas in the presence of moisture" will destroy most, if not all, of the pathogenic bacteria in the absence of spores. Four pounds of sulphur burned for each 1000 cubic feet will give an excess of gas.

Peroxide of hydrogen (H_2O_2) is an energetic disinfectant, and in 2 per cent. solution (about 40 per cent. of the ordinary commercial article) will kill the spores of anthrax in from two to three hours. A 20 per cent. solution of a good commercial hydrogen peroxide solution will quickly destroy the pyogenic cocci and other spore-free bacteria. It combines with organic matter, becoming inert. It is prompt in its action and not poisonous, but apt to deteriorate if not properly kept.

Chlorine.—Chlorine is a powerful gaseous germicide, owing its activity to its affinity for hydrogen and the consequent release of nascent oxygen when it comes in contact with micro-organisms in a moist condition. It is, therefore, a much more active germicide in the presence of moisture than in a dry condition. Thus, Fischer and Proskauer found that dried anthrax spores exposed for an hour in an atmosphere containing 44.7 per cent. of dry chlorine were not destroyed; but if the spores were previously moistened and were exposed in a moist atmosphere for the same time, 4 per cent. was effective, and when the time was extended to three hours 1 per cent. destroyed their vitality. The anthrax bacillus, in the absence of spores, was killed by exposure in a moist atmosphere containing 1 part to 2500 for twenty-four hours.

In watery solutions 0.2 per cent. kills spores within five minutes and the vegetative forms almost immediately.

Chloride of Lime.—The efficacy of chloride of lime depends on the chlorine it contains in the form of hypochlorites. A solution in water of 0.5 to 1 per cent. of chloride of lime will kill most bacteria in one to five minutes. A 5 per cent. solution usually destroys spores within one hour.

Bromine and iodine are of about the same value as chlorine for gaseous disinfectants, in the moist condition; but, like chlorine, they are not applicable for general use in house disinfection, owing to their poisonous and destructive properties; they have a use in sewers and similar places.

Trichloride of iodine in 0.5 per cent. solution destroys the vegetative forms of bacteria in five minutes.

Organic Disinfectants.

Alcohol in 10 per cent. solution inhibits the growth of bacteria; absolute alcohol kills bacteria in the vegetative form in from several to twenty-four hours.

Formaldehyde.—Formaldehyde, or formic aldehyde, was isolated by von Hoffmann in 1867, who obtained it by passing the vapors of methyl-alcohol mixed with air over finely divided platinum heated to redness. The methyl-alcohol is oxidized and produces formaldehyde as follows:

$$CH_2OH + O = CH_2O - H_2O.$$

Formaldehyde is a gaseous compound having the chemical formula CH_2O and possessed of an extremely irritating odor. At a temperature of 68° F. the gas is polymerized—that is to say, a second body is formed,

composed of a union of two molecules of CH_2O. This is known as a paraformaldehyde, and is a white, soapy body, soluble in boiling water and alcohol; it exists in the solution of commerce—a clear, watery liquid containing from 33 to 40 per cent. of the gas and 10 to 20 per cent. of methyl-alcohol, its chief impurity. If the commercial solution—ordinarily known in the trade as "formalin"—is evaporated or concentrated above 40 per cent., paraformaldehyde results; and when this is dried *in vacuo* over sulphuric acid a third body—trioxymethylene—is produced, consisting of three molecules of CH_2O. This is a white powder, almost soluble in water or alcohol, and giving off a strong odor of formaldehyde. The solid polymers of formaldehyde, when heated, are again reduced to the gaseous condition; ignited, they finally take fire and burn with a blue flame, leaving but little ash.

Formaldehyde has an active affinity for many organic substances, and forms with some of them definite chemical combinations. It combines readily with ammonia to produce a compound called ammoniacal aldehyde, which possesses neither the odor nor the antiseptic properties of formaldehyde. This action is made use of in neutralizing the odor of formaldehyde when it is desired to dispel it rapidly after disinfection. Formaldehyde also forms combinations with certain aniline colors—viz., fuchsin and safranin—the shades of which are thereby changed or intensified. These are the only colors, however, which are thus affected, and as they are seldom used in dyeing, owing to their liability to fade, this effect is of little practical significance. The most delicate fabrics of silk, wool, cotton, fur, leather, etc., are unaffected in texture or color by formaldehyde. Iron and steel are attacked, after long exposure, by the gas, and more so by its solution; but copper, brass, nickel, zinc, silver, and gilt work are not at all acted upon. Formaldehyde unites with nitrogenous products of decay—fermentation or decomposition—forming true chemical compounds, which are odorless and sterile. It is thus a true deodorizer in that it does not replace one odor by another more powerful, but forms new chemical compounds which are odorless. Formaldehyde has a peculiar action upon albumin, which it transforms into an insoluble and indecomposable substance. It renders gelatin insoluble in boiling water and most acids and alkalies. It is from this property of combining chemically with the albuminoids forming the protoplasm of bacteria that formaldehyde is supposed to derive its bactericidal powers. Formaldehyde is an excellent preservative of organic products. It has been proposed to make use of this action for the preservation of meat, milk, and other food products; but, according to Trillat and other investigators, formaldehyde renders these substances indigestible and unfit for food. It has been successively employed as a preservative of pathological and histological specimens.

There are no exact experiments recorded of the physiological action of formaldehyde on the human subject when taken internally. Slater and Rideal[1] report that a 1 per cent. solution has been taken in considerable

[1] Lancet, April 21, 1894.

quantity without serious results; and trioxymethylene has been given in doses up to 90 grains as an intestinal antiseptic. The vapors of formaldehyde are extremely irritating to the mucous membrane of the eyes, nose, and mouth, causing profuse lacrymation, coryza, and flow of saliva. Aronson reports that in many of his experiments rabbits and guinea-pigs allowed to remain for twelve and twenty-four hours in rooms which were being disinfected with formaldehyde gas were found to be perfectly well when the rooms were opened. On autopsy the animals showed no injurious effects of the gas. Others have noticed that animals, such as dogs and cats, which have accidentally been confined for any length of time in rooms undergoing formaldehyde disinfection occasionally died from the effects of the gas. Many observers, however, have reported that insects, such as roaches, flies, and bedbugs, are not, as a rule, affected. The result of these observations would seem to indicate that although formaldehyde is comparatively non-toxic to the higher forms of animal life, nevertheless a certain degree of caution should be observed in the use of this agent.

The results of numerous experiments have shown that in the air 2.5 per cent. by volume of the aqueous solution, or 1 per cent. by volume of the gas, are sufficient to destroy fresh virulent cultures of the common pathogenic bacteria in a few minutes. The researches of Pottevin and Trillat have shown that the germicidal power of the gas depends not only upon its concentration, but also upon the temperature and the condition of the objects to be sterilized. As with other gaseous disinfectants—viz., sulphur dioxide and chlorine—it has been found that the action is more rapid and complete at higher temperatures—*i. e.*, at 35° to 45° C. (95° to 120° F.)—and when the test objects are moist than at lower temperatures and when the objects are dry. Still, it has been repeatedly demonstrated by actual experiment in rooms that it is possible to disinfect the surface of apartments and articles contained in them, under the conditions of temperature and moisture ordinarily existing in rooms even in winter, by an exposure of a few hours to a saturated atmosphere of formaldehyde gas.

Stahl has shown that bandages and iodoform gauze can be kept well sterilized by placing in the jars containing them pieces of a preparation of paraformaldehyde in tablet form containing 50 per cent. of formaldehyde. The same experimenter has also succeeded in making carpets and articles of clothing germ-free by spraying them with 0.5 to 2 per cent. solution of formaldehyde for fifteen to twenty minutes without the color of the fabrics being in any way affected. The investigations of Trillat, Aronson, Pottevin, and others have shown that a concentration of $\frac{1}{10000}$ of the aqueous solution (40 per cent.), equal to $\frac{1}{25000}$ of pure formaldehyde, was safe and sufficiently powerful to retard bacterial growth.

A 2 per cent. watery solution of formalin destroys the vegetative forms of bacteria within five minutes. In our experiments formalin has upon the vegetative forms about one-half the strength of pure carbolic acid.

Chloroform.—This substance, even in pure form, does not destroy spores, but it does bacteria in vegetative form, even in 1 per cent. solution. Chloroform is used practically in sterilizing and keeping sterile blood serum, which can be used later for culture purposes by driving off the chloroform.

Iodoform.—This substance has but very little destructive action upon bacteria; indeed, upon most varieties it has no appreciable effect whatever. When mixed with putrefying matter, wound discharges, etc., the iodoform is reduced into soluble iodine compounds, which partly act destructively upon the bacteria and partly unite with the poisons already produced.

Carbolic Acid (C_6H_5OH).—A solution having 1 part to 1000 inhibits the growth of bacteria; 1 part to 400 kills the less resistant bacteria, and 1 part to 100 kills the remainder. A 5 per cent. solution kills the less resistant spores within a few hours and the more resistant in from one day to four weeks. A slight increase in temperature aids the destructive action; thus, even at 37.5° spores are killed in three hours. A 3 per cent. solution kills streptococci, staphylococci, anthrax bacilli, etc., within one minute. Carbolic acid loses much of its value when in solution in alcohol or ether. An addition of 0.5 HCl aids its activity. Carbolic acid is so permanent and so comparatively little influenced by albumin that it is rightly widely used in practical disinfection even in places of more powerful substances.

Cresol.—Cresol [$C_6H_4(CH_3)OH$] is the chief ingredient of the so-called "crude carbolic acid." This is almost insoluble in water, and therefore of restricted value. Many methods are used for bringing it into solution so as to make use of its powerful disinfecting properties. With equal parts of crude sulphuric acid it is a powerful disinfectant, but it is, of course, strongly corrosive. An alkaline emulsion of the cresols and other products contained in "crude" carbolic acid with soap is called creolin. It is used in 1 to 5 per cent. emulsions. It is fully as powerful as pure carbolic acid. Lysol is similar to creolin, except that it has more of the cresols and less of the other products. It and creolin are of about the same value.

Tricresol.—Tricresol is a refined mixture of the three cresols (metacresol, paracresol, and orthocresol). It is soluble in water to the extent of 2.5 per cent., and is about three times the strength of carbolic acid.

Aniline Dyes.—Some of these colors possess marked germicidal qualities. According to observers, methyl violet (pyoktanin) and malachite green destroy the typhoid bacillus in bouillon cultures in the proportion of 1:200 in two hours' exposure, and the pyogenic cocci in less. In 1:100,000 solutions they are said to retard the development of bacteria.

Oil of turpentine, 1:200, prevents the growth of bacteria.

Camphor has very slight antiseptic action.

Creosote in 1:200 kills many bacteria in ten minutes; 1:100 failed to kill tubercle bacilli in twelve hours.

Essential oils: Cardéac and Meumir found that the essences of cinnamon, cloves, thyme, and others killed typhoid bacilli within one hour. Sandalwood required twelve hours.

Thymol and eucalyptol have about one-fourth the strength of carbolic acid (Behring).

Oil of peppermint in 1:100 solution prevents the growth of bacteria.

TABLE OF ANTISEPTIC VALUES.[1]

Alum	1 : 222	Mercuric chloride	1 : 14,300
Aluminum acetate	1 : 6000	Mercuric iodide	1 : 40,000
Ammonium chloride	1 : 9	Potassium bromide	1 : 10
Boric acid	1 : 143	Potassium iodide	1 : 10
Calcium chloride	1 : 25	Potassium permanganate	1 : 300
Calcium hypochlorite	1 : 1000	Pure formaldehyde	1 : 25,000
Carbolic acid	1 : 333	Quinine sulphate	1 : 800
Chloral hydrate	1 : 107	Silver nitrate	1 : 12,500
Cupric sulphate	1 : 2000	Sodium borate	1 : 14
Ferrous sulphate	1 : 200	Sodium chloride	1 : 6
Formaldehyde (40 %)	1 : 10,000	Zinc chloride	1 : 500
Hydrogen peroxide	1 : 20,000	Zinc sulphate	1 : 20

[1] These figures are approximately correct, and represent the percentage of disinfectant required to be added to a fluid containing considerable organic material, in order to permanently inhibit any bacterial growth.

CHAPTER X.

PRACTICAL DISINFECTION AND STERILIZATION (HOUSE, PERSON, INSTRUMENTS, AND FOOD)—STERILIZATION OF MILK FOR FEEDING INFANTS.

Disinfectants and Methods of Disinfection Employed in the House and Sick-room.

Disinfection and Disinfectants.—Sunlight, pure air, and cleanliness are always very important agents in maintaining health and in protecting the body against many forms of illness. When, however, it becomes necessary to guard against such special dangers as accumulated filth or contagious diseases, disinfection is essential. In order that disinfection shall afford complete protection it must be thorough; and perfect cleanliness is better, even in the presence of contagious disease, than filth with poor disinfection.

Since all forms of fermentation, decomposition, and putrefaction, as well as the infectious and contagious diseases, are caused by micro-organisms, it is the object of disinfection to kill these. Decomposition and putrefaction should at all times be prevented by the immediate destruction or removal from the neighborhood of the dwelling of all useless putrescible substances. In order that as few articles as possible shall be exposed to the germs causing the contagious diseases, and thus become carriers of infection, it is important that all articles not necessary for immediate use in the care of the sick person, especially upholstered furniture, carpets, and curtains, should be removed from the room before placing the sick person in it.

Agents for Cleansing and Disinfection.

Too much emphasis cannot be placed upon the importance of cleanliness, both as regards the person and the dwelling, in preserving health and protecting the body from all kinds of infectious disease. Sunlight and fresh air should be freely admitted through open windows, and personal cleanliness should be attained by frequently washing the hands and body.

Cleanliness in dwellings, and in all places where men go, may, under ordinary circumstances, be well maintained by the use of the two following solutions:

1. **Soapsuds Solution.**—For simple cleansing, or for cleansing after the method of disinfection by chemicals described below, one ounce of common soda should be added to twelve quarts of hot soapsuds (soft soap and water).

2. **Strong Soda Solution.**—This, which is a stronger and more effective cleansing solution and also a feeble disinfectant, is made by dissolving one-half pound of common soda in three gallons of hot water. The solution thus obtained should be applied by scrubbing with a hard brush.

When it becomes necessary to arrest putrefaction or to prevent the spread of contagious diseases by surely killing the living germs which cause them, more powerful agents must be employed than those required for simple cleanliness, and these are commonly called disinfectants. The following are some of the most reliable ones:

3. **Heat.**—Complete destruction by fire is an absolutely safe method of disposing of infected articles of small value, but continued high temperatures not as great as that of fire will destroy all forms of life; thus, boiling or steaming in closed vessels for one-half hour will absolutely destroy all disease germs.

4. **Carbolic Acid Solution.**—Dissolve six ounces of carbolic acid in one gallon of hot water. This makes approximately a 5 per cent. solution of carbolic acid, which, for many purposes, may be diluted with an equal quantity of water. The commercial "soluble crude carbolic acid" can be used instead of the pure carbolic acid for privies and drains. It makes a white emulsion on account of its not entering readily into solution. Care must be taken that the pure acid does not come in contact with the skin.

5. **Bichloride Solution** (bichloride of mercury or corrosive sublimate).—Dissolve sixty grains of pulverized corrosive sublimate and two tablespoonfuls of common salt in one gallon of hot water. This solution, which is approximately 1:1000, must be kept in glass, earthen, or wooden vessels (not in metal vessels). For safety it is well to cover the solution.

The carbolic and bichloride solutions are very poisonous when taken by the mouth, but are harmless when used externally.

6. **Milk of Lime.**—This mixture is made by adding one quart of dry, freshly slaked lime to four or five quarts of water. (Lime is slaked by pouring a small quantity of water on a lump of quicklime. The lime becomes hot, crumbles, and as the slaking is completed a white powder results. The powder is used to make milk of lime.) Air-slaked lime (the carbonate) has no value as a disinfectant.

7. **Dry "Chloride of Lime."**—This must be fresh and kept in closed vessels or packages. It should have the strong, pungent odor of chlorine.

8. **Formalin.**—Add 1 part of formalin to 10 of water. This equals in value the 5 per cent. carbolic acid solution.

9. **Creolin, tricresol, and lysol** are of about the same value as pure carbolic acid.

The proprietary disinfectants, which are so often widely advertised and whose composition is kept secret, are relatively expensive and often unreliable and inefficient. It is important to remember that substances which destroy or disguise bad odors are not necessarily disinfectants, and that there are very few disinfectants that are not poisonous when taken internally.

[NOTE.—The cost of the pure carbolic acid solution is much greater than that of most of the other solutions, but except for the disinfection of the skin, which in some persons it irritates, and of woodwork, it is generally much to be preferred by those not thoroughly familiar with disinfectants, as it does not deteriorate, and is rather more uniform in its action than some of the other disinfectants.]

Methods of Disinfection in Infectious and Contagious Diseases.

The diseases to be commonly guarded against, outside of surgery, by disinfection are scarlet fever, measles, diphtheria, tuberculosis, smallpox, typhoid and typhus fever, bubonic plague and cholera.

1. **Hands and Person.**—Dilute the carbolic solution with an equal amount of water or use the bichloride solution without dilution. Hands soiled in caring for persons suffering from contagious diseases, or soiled portions of the patient's body, should be immediately and thoroughly washed with one of these solutions and then washed with soap and water, and finally immersed again in the solutions. The nails should always be kept perfectly clean. Before eating, the hands should be first washed in one of the above solutions, and then thoroughly scrubbed with soap and water by means of a brush.

2. **Soiled clothing, towels, napkins, bedding, etc.**, should be immediately immersed in the carbolic solution, in the sick-room, and soaked for one or more hours. They should then be wrung out and boiled in the soapsuds solution for one hour. Articles such as beds, woollen clothing, etc., which cannot be washed, should at the end of the disease be referred to the Health Department, if such is within reach, for disinfection or destruction; or if there is no public disinfection, these goods should be thoroughly exposed to formaldehyde gas, as noted later.

3. **Food and Drink.**—Food thoroughly cooked and drinks that have been boiled are free from disease germs. Food and drinks, after cooking or boiling, if not immediately used, should be placed when cool in clean dishes or vessels and covered. In the presence of an epidemic of cholera or typhoid fever, milk and water used for drinking, cooking, washing dishes, etc., should be boiled before using, and all persons should avoid eating uncooked fruit and fresh vegetables. Instead of boiling milk may be heated to 80° C. for one-half hour.

4. **Discharges of all kinds from the mouth, nose, bladder, and bowels** of patients suffering from contagious diseases should be received into glass or earthen vessels containing the carbolic solution, or milk of lime, or they should be removed on pieces of cloth, which are immediately immersed in one of these solutions. Special care should be observed to disinfect at once the vomited matter and the intestinal discharges from cholera patients. In typhoid fever the urine and the intestinal discharges, and in diphtheria, measles, and scarlet fever the discharges from the throat and nose all carry infection and should be treated in the same manner. The volume of the solution used to disinfect discharges should be at least twice as great as that of the discharge, and

should completely mix with and cover it. After standing for an hour or more the disinfecting solution with the discharges may be thrown into the water-closet. Cloths, towels, napkins, bedding, or clothing soiled by the discharges must be at once placed in the carbolic solution, and the hands of the attendants disinfected, as described above. In convalescence from measles and scarlet fever the scales from the skin are also carriers of infection. To prevent the dissemination of disease by means of these scales the skin should be carefully washed daily in warm soap and water. After use the soapsuds should be disinfected and thrown into the water-closet.

Masses of feces are extremely difficult to disinfect except on the surface, for it takes disinfectants such as the carbolic acid solution some twelve hours to penetrate to their interior. If fecal masses are to be thrown into places where the disinfectant solution covering them will be washed off, it will be necessary to be certain that the disinfectant has previously penetrated to all portions and destroyed the disease germs. This can be brought about by stirring them with the disinfectant and allowing the mixture to stand for one hour, or by washing them into a pot holding soda solution which is already at the boiling temperature, or later will be brought to one.

5. **Sputum from Consumptive Patients.**—The importance of the proper disinfection of the sputum from consumptive patients is still underestimated. Consumption is an infectious disease, and is always the result of transmission from the sick to the healthy or from animals to man. The sputum contains the germs which cause the disease, and in a large proportion of cases is the source of infection. After being discharged, unless properly disposed of, it may become dry and pulverized and float in the air as dust. This dust contains the germs, and is a common cause of the disease, through inhalation. In all cases, therefore, the sputum should be disinfected when discharged. It should be received in covered cups containing the carbolic or milk-of-lime solution. Handkerchiefs soiled by it should be soaked in the carbolic solution and then boiled. Dust from the walls, mouldings, pictures, etc., in rooms that have been occupied by consumptive patients, where the rules of cleanliness have not been carried out, contain the germs and will produce tuberculosis in animals when used for their inoculation; therefore, rooms should be thoroughly disinfected before they are again occupied. If the sputum of all consumptive patients were destroyed at once when discharged a large proportion of the cases of the disease would be prevented.

6. **Closets, Kitchen and Hallway Sinks, etc.**—The closet should never be used for infected discharges until they have been thoroughly disinfected, if it can be avoided; if done, one pint of carbolic solution should be poured into the pan (after it is emptied) and allowed to remain there. Sinks should be flushed at least once daily.

7. **Dishes, knives, forks, spoons, etc.**, used by a patient should, as a rule, be kept for his exclusive use and not removed from the room. They should be washed first in the carbolic solution, then in boiling

hot soapsuds, and finally rinsed in hot water. These washing fluids should afterward be thrown into the water-closet. The remains of the patient's meals may be burned or thrown into a vessel containing the carbolic solution or milk of lime, and allowed to stand for one hour before being thrown away.

8. **Rooms and Their Contents.**—Rooms which have been occupied by persons suffering from contagious disease should not be again occupied until they have been thoroughly disinfected. For this purpose either careful fumigation with formaldehyde gas or sulphur should be employed, or this combined with the following procedure: Carpets, curtains, and upholstered furniture which have been soiled by discharges, or which have been exposed to infection in the room during the illness, will be removed for disinfection to chambers where they can be exposed to formaldehyde gas and moderate warmth for twelve to twenty-four hours, or to steam. Some carpets, such as many Wiltons, are discolored by moist steam. These must be put in the formaldehyde chamber. Woodwork, floors, and plain furniture will be thoroughly washed with the soapsuds and bichloride solutions.

9. **Rags, cloths, and articles of small value,** which have been soiled by discharges or infected in other ways, should be boiled or burned.

10. **In case of death** the body should be completely wrapped in several thicknesses of cloth wrung out of the carbolic or bichloride solution, and when possible placed in an hermetically sealed coffin.

It is important to remember that *an abundance of fresh air, sunlight, and absolute cleanliness* not only helps protect the attendants from infection and aid in the recovery of the sick, but directly destroys the bacteria which cause disease.

Methods of Cleanliness and Disinfection to Prevent the Occurrence of Illness.

1. **Water-closet bowls and all receptacles for human excrement** should be kept perfectly clean by frequent flushing with a large quantity of water, and as often as necessary disinfected with the carbolic, bichloride, or other efficient solutions. The woodwork around and beneath them should be frequently scrubbed with the hot soapsuds solution.

2. **Sinks and the woodwork around and the floor beneath them** should be frequently and thoroughly scrubbed with the hot soapsuds solution.

3. **School Sinks.**—School sinks should be thoroughly flushed with a large quantity of water at least twice daily, and should be carefully cleaned twice a week or oftener by scrubbing. Several quarts of the carbolic solution should be frequently thrown in the sink after it has been flushed.

4. **Cesspools and Privy Vaults.**—An abundance of milk of lime or chloride of lime should be thrown into these daily, and their contents should be frequently removed.

5. **Cellars and rooms in cellars** are to be frequently whitewashed, and, if necessary, the floors sprinkled with dry chloride of lime. *Areas and*

paved yards should be cleaned, scrubbed, and, if necessary, washed with the bichloride solution. *Street gutters and drains* should be cleaned and, when necessary, sprinkled with chloride of lime or washed with milk of lime.

6. **Air-shafts.**—Air-shafts should be first cleaned thoroughly and then whitewashed. To prevent tenants throwing garbage down air-shafts it is sometimes advisable to put wire netting outside of windows opening on shafts. Concrete or asphalt bottoms of shafts should be cleaned and washed with the bichloride solution or sprinkled with chloride of lime.

7. **Hydrant sinks, garbage receptacles, and garbage and oyster-shell chutes and receptacles** should be cleaned daily and sprinkled with dry chloride of lime.

8. **Refrigerators and the surfaces around and beneath them, dumb-waiters, etc.,** may be cleaned by scrubbing them with the hot soapsuds solution.

9. **Traps.**—All traps should be flushed daily with an abundance of water. If at any time they become foul they may be cleaned by pouring considerable quantities of the hot strong soda solution into them, followed by the carbolic solution.

10. **Urinals and the floors around and beneath them** should be cleaned twice daily with the hot soapsuds solution, and in addition to this, if offensive, they may be disinfected with the carbolic solution.

11. **Stable Floors and Manure Vaults.**—Stable floors should be kept clean and occasionally washed with the hot soapsuds or the hot strong soda solution. Powdered fresh chloride of lime or formalin may be used in manure vaults.

12. **Vacant rooms** should be frequently aired.

13. **The woodwork in school-houses** should be scrubbed weekly with hot soapsuds. This refers to floors, doors, door-handles, and all woodwork touched by the scholars' hands.

14. **Spittoons in all public places** should be emptied daily and washed with the hot soapsuds solution, after which a small quantity of the carbolic solution or milk of lime should be put in the vessel to receive the expectoration.

15. **Cars, Ferry-boats, and Public Conveyances.**—The floors, door-handles, railings, and all parts touched by the hands of passengers should be washed frequently with the hot soapsuds solution. Slat-mats from cars, etc., should be cleaned by scrubbing with a stiff brush in the hot soapsuds solution.

Telephone receiver mouth-pieces should also be frequently cleansed.

Use of Bromine Solution as a Deodorant.—*Slaughter-houses, butchers' ice-boxes and wagons, trenches, excavations, stable floors, manure-vaults, dead animals, offal, offal docks, etc.,* may be deodorized by a weak solution of bromine, which is a valuable agent for this purpose. The bromine solution, however, is only temporary in its action, and must be used repeatedly. It should be applied by sprinkling. Although somewhat corrosive in its action on metals, it is otherwise harmless.

The solution of bromine must be prepared with great care, as the pure bromine from which it is made is dangerous. It is very caustic when brought in contact with the skin; it is volatile and its fumes are very irritating when inhaled. To prepare the solution an ounce bottle of liquid bromine is dropped into three gallons of water, and broken under the water and thoroughly stirred.

The Practical Employment of Formaldehyde and Sulphur Dioxide Gases in the Surface Disinfection of Rooms and the Disinfection of Goods which would be Injured by Heat.—Formaldehyde gas has come into such general use, and is for many purposes so valuable, that the description of methods employed to generate and use it will be given in detail.

If we consider now the practical application of formaldehyde gas for purposes of disinfection we find that its destructive action on micro-organisms depends upon a number of factors, the chief of which are its concentration in the surrounding atmosphere, the length of the contact, the existing temperature, the accompanying moisture, and the nature of the organism.

The necessary concentration of the gas in the surrounding atmosphere to kill the micro-organisms varies with each species, for some resist chemical agents much more than others, and also with the freedom of access of the gas to the bacteria, for if they are under cover or within fabrics a greater amount of gas must be generated than if they are freely exposed.

For purely surface disinfection, when the less resistant bacteria or other micro-organisms are to be destroyed, there will be required, according to the method used, six to ten ounces of formalin of full strength, or its equivalent, to 1000 cubic feet.

For the destruction of the more resistant but non-spore bearing forms, such as typhoid fever or tubercle bacilli, at least twelve ounces of formalin should be used. The gas penetrates through fabrics with difficulty, and to pass through heavy goods the concentration of the gas must be doubled and moderate heat added (45 C.° or above).

Value of Moisture.—At first it was thought that formaldehyde gas acted more effectually in a dry atmosphere, but further investigation has proved that, although it does destroy bacteria with the amount of moisture usually present in the air, and contained in their own substance, it acts much more powerfully and certainly when additional moisture is present, and best when present up to the point of saturation. The actual spraying of walls and goods to be disinfected with water is even more efficacious.

A fairly high temperature—but one still below that which would injure delicate fabrics—increases not only the activity of formaldehyde gas but also its penetrative power, and for heavy goods it is essential. The production of a partial vacuum in the chambers before the introduction of the folmaldehyde gas still further assists its penetration.

The length of exposure necessary for complete disinfection depends upon the nature of the disease for which it is carried out—the penetra-

tion required, the concentration of the gas used, the amount of moisture in the air, the temperature of the air, and the size and shape of the room. For surface disinfection in rooms, when as much as twelve ounces of formalin are used for each 1000 cubic feet, five hours' exposure is amply sufficient, most bacteria being killed within the first few minutes. For the destruction of micro-organisms protected by even a layer of thin covering, double the formalin and double the time of exposure should be allowed, and even then the killing of many species of non-spore bearing bacteria cannot be counted upon in ordinary rooms. When absolutely complete disinfection is demanded, where penetration of gas is required, the goods must be placed in chambers where moderate heat can be added and all leakage of gas prevented.

Various forms of apparatus can be properly employed to liberate formaldehyde gas for purposes of disinfection. There are two essentials to any good method—namely, that the formaldehyde gas is given off quickly, and that there is no great loss by deterioration of the formalin.

Wood Alcohol.—A number of lamps have been devised, all very much on the same principle, though varying somewhat in mechanical construction, which bring about the incomplete oxidation of methyl-alcohol by passing the vapors mixed with air over the incandescent metal. Although disinfection can be carried out by the best of these lamps, in our experience none of them up to the present time are satisfactory or economical. They may be very useful as deodorizers in the sick-room or other places.

The same principle is used efficiently in another form. The vapor of wood alcohol is passed over surfaces of asbestos containing particles of finely divided platinum. This apparatus has given very good results, and for a given amount of disinfection leaves less odor of formaldehyde gas in the room than any other. The apparatus is, however, bulky and expensive.

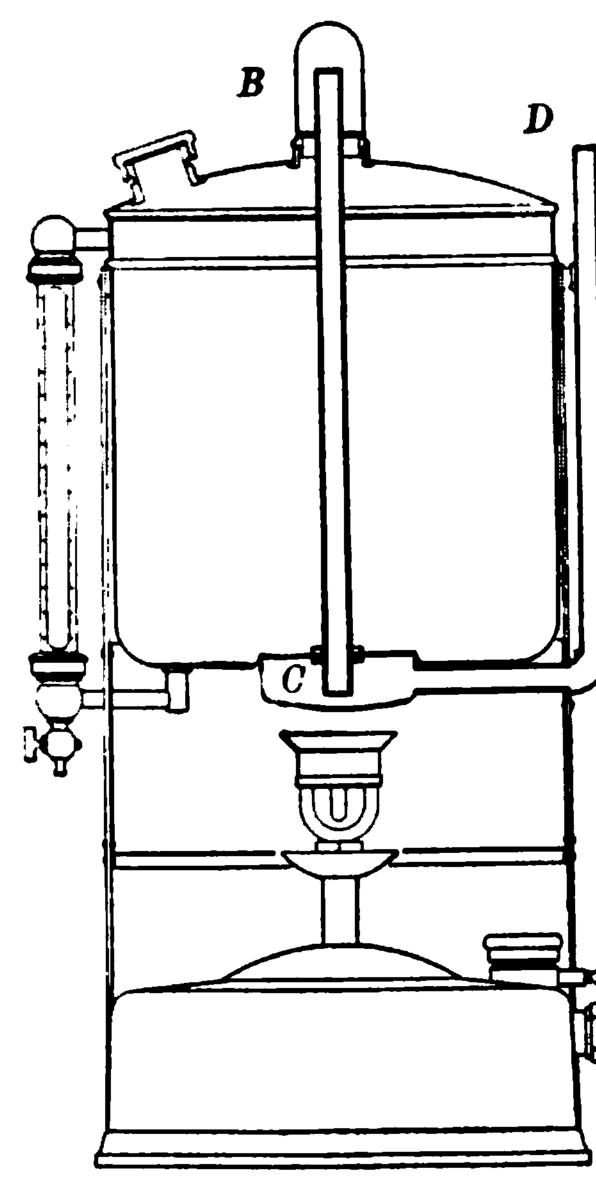
Formochloral by the Trillat System.—This system consists in heating, under three atmospheres of pressure, a solution of formaldehyde gas in water mixed with 30 per cent. of calcium chloride, known as "formochloral," to a temperature of 135° C. (275° F.). It is claimed for this method of producing the gas from formochloral that the polymerization of the formaldehyde is prevented, which would otherwise take place if a solution of formaldehyde were evaporated under ordinary conditions, and that thereby the whole of the formaldehyde is obtained in the gaseous state. The addition of any neutral salt aids the process, it is said, but calcium chloride is the best. The results with this apparatus have been satisfactory, but not more so than by other methods. The apparatus is expensive and heavy and therefore unnecessary.

Formalin by Boiling and Passing the Vapor through a Superheated Coil or Chamber.—This system consists in heating the ordinary commercial formalin to a temperature of about 260° C. (500° F.) in an incandescent copper coil or chamber, and allowing the vapors to pass off freely. It is claimed for this method that the degree of heat necessary to break up the polymerized products formed is supplied, and thus a loss of

formaldehyde is prevented. A further action of the intense heat in the copper tube on the solution is to partially convert the methyl-alcohol contained in commercial formalin into formaldehyde gas by partial oxidation, thereby preventing the formation of methyl and increasing the amount of formaldehyde.

The apparatus consists of a closed receiver of copper holding about a gallon, a coil of copper pipe attached at one end to the bottom of the receiver, and, like the preceding apparatus and that made by Lentz, at the other, by means of a suitable connection (rubber tube with gutta-percha or metallic mouth-piece), with the room or apartment to be disinfected, and a heating lamp (Swedish lamp or Bunsen burner). In operation the desired quantity of formalin is placed in the receiver and the receiver is closed. The lamp is lighted and the coil brought to a red heat. The valve is then opened and the solution contained in the receiver is allowed to pass down and into the coil in a fine stream. Upon coming in contact with the heated metal the formaldehyde solution is instantly decomposed, and the liberated gas is further purified as it progresses through the incandescent coil. The apparatus is liable to get out of order, in that the valve is apt to become clogged and so stop the flow of formalin until freed by a wire supplied for the purpose.

FIG. 65



Formaldehyde apparatus.

In the new form (Fig. 65) the formalin is first boiled in the large chamber and passes as vapor through the tube connecting *B* and *C*. In *C* it is superheated and passes out the tube *D* into the room. In this apparatus there is nothing to get out of order, and it operates quickly. Up to the present time this is the most practical apparatus we have met with, when the initial cost, about $25, is not an objection. In all forms of apparatus where formalin is used the large receiving chamber should be washed out from time to time with hot water, to remove any deposit there may be.

Trioxymethylene by Schering's System.—This system consists in heating the solid polymer of formaldehyde (trioxymethylene) in a lamp specially constructed for the purpose by the Chemische Fabrik auf Actien, in Berlin. The trioxymethylene is used in the form of compressed tablets or pastilles, as being more convenient for use. Each pastille contains the equivalent of 100 per cent. of formaldehyde gas, according to the manufacturers, and weighs 1 gram.

The mode of using the apparatus is very simple: The disinfector is placed upon a sheet of iron on the floor of the room to be disinfected. From 100 to 250 pastilles can be evaporated at a time in the apparatus.

For the production of greater quantities of formaldehyde vapor several of these outfits may be used together. The lamp is filled with ordinary or wood alcohol, about twice as many cubic centimetres of the alcohol being employed as there are pastilles to be evaporated. The wicks should project but little above the necks of the burners, or the apparatus may get too hot and ignite the pastilles. The vessel is charged with formalin pastilles and the disinfector placed over the lighted spirit lamp. The lamp is then allowed to burn out in the closed room. One hundred pastilles are considered to be sufficient for the disinfection of 1000 cubic feet of space. Lately, a small steam boiler has been added to the apparatus, for the purpose of furnishing sufficient moisture with the gas. The results obtained by us in superficial disinfection, when from 150 to 200 pastilles have been used to each 1000 cubic feet, have been good. The great advantage of the method is in the small cost of the apparatus, $3.00, and the avoidance of the danger of deterioration, which is present to some extent in formalin. Smaller lamps are very useful for the deodorization of rooms.

From Pastilles Composed of a Top of Compressed Paraform and a Base of Prepared Charcoal.—This is a very neat but somewhat expensive method of liberating formaldehyde gas. Our results with it have been good.

Formalin to which Glycerin has been Added.—To the formalin is added 10 per cent. of glycerin, and the mixture is simply boiled in a suitable copper vessel, the steam and formaldehyde gas passing off by a tube. This is a very serviceable apparatus. When it is attempted to vaporize the formalin too rapidly part of it passes over in fluid form, and is thus wasted.

With a slightly greater amount of formalin than that used in the high temperature autoclave and heated tube or chamber methods, the results seem to be equally as good. The apparatus is very easy to use, and is not liable to get out of order.

Similar forms of apparatus are also employed, when instead of glycerin the formalin is mixed with an equal quantity of water. The water is for the purpose of giving additional moisture to the air, and, at the same time, like the glycerin, to prevent the change of formaldehyde into inert substances.

From Formalin in an Open Pan.—A very simple method, devised by Dr. R. J. Wilson, is to fill a tin pan with twelve ounces of formalin for each 1000 cubic feet and put this on an upright sheet of tin, which is cut so as to allow of the entrance of air below and yet protect the formalin in the pan from the flame. For heating put under it a small tin can filled with asbestos packing which has been soaked with wood alcohol. A still simpler method is to hang sheets in a room and throw on them twelve ounces of formalin for each 1000 cubic feet, and leave for ten hours. If the room is tightly sealed very fair superficial disinfection will take place.

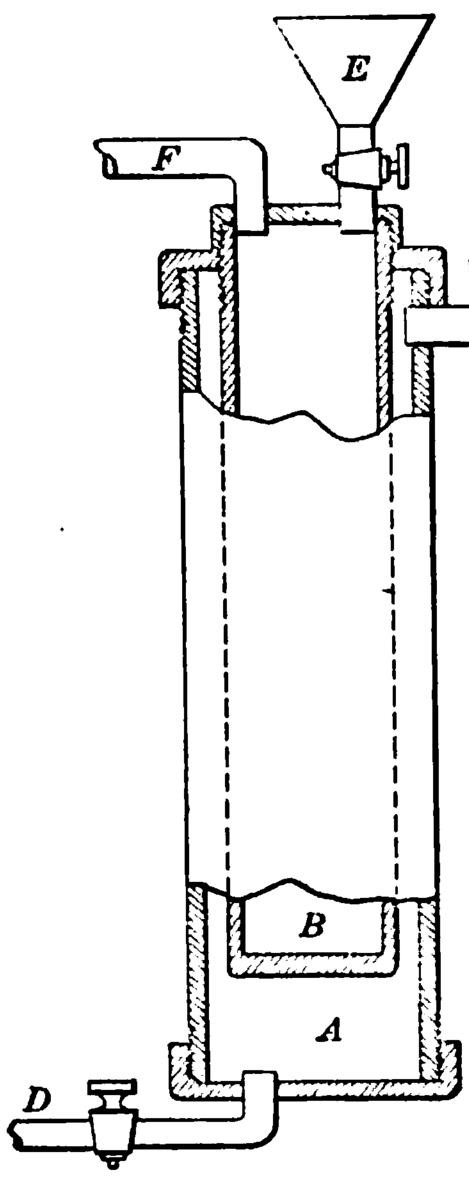
Lime Method of Generating Formaldehyde Gas.—The use of quicklime for generating formaldehyde gas has been practised by various

observers, with varying results. The differing results can probably be explained by difference in technique and in the kind of lime used. It is absolutely necessary to have a quick slaking lime or a great amount of the gas will be lost by polymerization into paraformaldehyde and acrose. Even with quick slaking lime, if it is not spread in a comparatively thin layer, polymerization takes place; therefore, in applying the method a wide pan must be used. The addition of concentrated sulphuric acid to the formaldehyde solution in the proportion of 10 per cent. immediately before using lessens the danger of polymerization and makes the evolution of the gas much more rapid. The sulphuric acid must not be added to the formaldehyde solution until just before using, for it causes rapid polymerization in the solution. It must be remembered, also, that sulphuric acid is a dangerous agent, and careless handling of it might result in serious burns. The technique of the method is as follows: To ten ounces of 40 per cent. formaldehyde solution slowly add one ounce of concentrated sulphuric acid; pour this solution on to two pounds of quicklime that has previously been cracked into small lumps and placed in a dairy pan not less than twelve inches in diameter. The liberation of a large amount of gas in a short time more than compensates for the loss by polymerization, and disinfection is effected by a quick union of the gas and organisms to be destroyed. Saturated solution of aluminum sulphate may be used instead of concentrated sulphuric acid, in the proportion of one part of aluminum sulphate solution to three parts of 40 per cent. formaldehyde solution. The mixture of aluminum sulphate and formaldehyde will stand for considerable time without polymerization. Good results have been obtained from pouring 40 per cent. solution of formaldehyde into commercial permanganate of potassium in the proportion of six ounces of permanganate for every pint of 40 per cent. solution of formaldehyde.

Rapid Generation of Formaldehyde Gas for Large Chambers by the Method of Dr. R. J. Wilson.—The generator is made of ordinary iron steam pipe and can be manufactured in any pipe-cutting establishment in a very few hours. It consists of an outer steam jacket of six-inch pipe, two feet long, and capped at both ends. Through the upper cap there is a four-inch opening, with a thread, through which projects an inner chamber for formalin. This chamber consists of a four-inch pipe, twenty-two inches long, capped at the upper end and welded or capped at the lower end. The upper end of this pipe is so threaded as to permit of its being screwed through the cap of the steam jacket before that cap is screwed on. The cap of the formalin chamber is fitted on the same thread that passes through the cap of the steam jacket. The in-take for steam is near the top of the steam jacket, through a half-inch pipe, and the steam is controlled by a globe valve. The outlet for steam or drip is through a half-inch pipe from the bottom cap of the chamber and is also controlled by a globe valve. The in-take for formalin is through the upper cap of the formalin chamber through a half-inch pipe controlled by a globe valve. The outlet for formaldehyde is a half-inch pipe through the upper cap of the formalin chamber.

This generator is cheap and efficient, but considerable care should be observed in operating it, as there is a tendency to throw out some formalin before the gas begins to be evolved. This is easily avoided by using care in the proper application of the heat. These generators have now been in use for three years by the New York Health Department, and have given complete satisfaction.

FIG. 66



A, steam chamber; *B*, formalin chamber; *C*, steam supply; *D*, drip; *E*, inlet for formalin; *F*, outlet for formaldehyde.

As a result of the investigations undertaken in the Department of Health laboratories on the use of formaldehyde as a disinfectant, and a consideration of the work of others, the conclusions reached by us may be summarized as follows:

1. Disinfection of Infected Dwellings.—Exposed surfaces of walls, carpets, hangings, etc., in rooms may be superficially disinfected by means of formaldehyde gas. All apertures in the rooms should be tightly closed and from six to twelve ounces of formalin or its equivalent used to generate the gas for each 1000 cubic feet. The time of exposure should be not less than four hours, and a suitable apparatus should be employed. The temperature of the apartment should be as high as possible, and certainly not below 50° F. With even lower temperature disinfection is possible, but larger amounts of formalin must be used. When generated very rapidly the formaldehyde gives much better results than when given off slowly.

Under these conditions spore-free bacteria and the contagion of the exanthemata are surely destroyed when freely exposed to the action of the gas. Spore-bearing bacteria are not thus generally destroyed; but these latter are of such rare occurrence in disease that in house disinfection they may usually be disregarded, and, if present, special measures can be taken.

The penetrative power of formaldehyde gas in the ordinary room, at the usual temperature, even when used in double the strength necessary for surface disinfection, is extremely limited, not passing, as a rule, through more than one layer of cloth of medium thickness. Articles, therefore, such as bedding, carpets, upholstery, clothing, and the like should, when possible, be subjected to steam, hot air, or formaldehyde disinfection in special chambers constructed for the purpose. If not, they must be thoroughly exposed on all sides.

2. Disinfection of Bedding, Carpets, Upholstery, etc.—Bedding, carpets, clothing, etc., which would be injured by steam, may be disinfected by means of formaldehyde gas in an ordinary steam disin-

fecting chamber, the latter to be provided with a heating and if possible a vacuum apparatus and special apparatus for generating the gas. Where penetration through heavy articles is required the gas should be used in the proportion of not less than the amount derived from thirty ounces of formalin for each 1000 cubic feet, the time of exposure to be not less than eight hours and the temperature of the chamber not below 110° F.

In order to ensure complete sterilization of the articles they should be so placed as to allow of a free circulation of the gas around them—that is, in the case of bedding, clothing, etc., these should either be spread out on perforated wire shelves or loosely suspended in the chamber. The aid of a partial vacuum facilitates the operation. Upholstered furniture and articles requiring much space should be placed in a large chamber, or, better, in a room which can be heated to the required temperature.

The most delicate fabrics, furs, leather, and other articles, which are injured by steam, hot air at 230° F., or other disinfectants, are unaffected by formaldehyde.

3. Disinfection of Books.—Books may be satisfactorily disinfected by means of formaldehyde gas in a special room, or in the ordinary steam chamber, as above described, and under the same condition of volume of gas, temperature, and time of exposure. The books should be arranged to stand as widely open as possible upon perforated wire shelves, set about one or one and a half feet apart in the chamber. A chamber having a capacity of 200 to 250 cubic feet would thus afford accommodation for about one hundred books at a time.

Books, with the exception of their surfaces, cannot be satisfactorily disinfected by formaldehyde gas in the bookcases of houses and libraries, or anywhere except in special chambers constructed for the purpose, because the conditions required for their thorough disinfection cannot otherwise be complied with.

The bindings, illustrations, and print of books are in no way affected by the action of formaldehyde gas.

4. Disinfection of Carriages, etc.—Carriages, ambulances, cars, etc., can easily be disinfected by having built a small, tight building, in which they are enclosed and surrounded with formaldehyde gas. Such a building is used for disinfecting ambulances in New York City. With the apparatus there employed a large amount of formalin is rapidly vaporized, and superficial disinfection is completed in sixty minutes.

5. Advantages of Formaldehyde Gas over Sulphur Dioxide for Disinfection of Dwellings.—Formaldehyde gas is superior to sulphur dioxide as a disinfectant for dwellings: first, because it is more efficient in its action; second, because it is less injurious in its effects on household goods; third, because when necessary it can easily be supplied from a generator placed outside of the room and watched by an attendant, thus avoiding in some cases danger of fire.

Apart from the cost of the apparatus and the greater time involved, formaldehyde gas, generated from commercial formalin, is not much

more expensive than sulphur dioxide—viz., fifteen to twenty cents per 1000 cubic feet against ten cents with sulphur. Therefore, we believe that formaldehyde gas is the best disinfectant at present known for the surface disinfection of infected dwellings. For heavy goods it is far inferior in penetrative power to steam; but for the disinfection of fine wearing apparel, furs, leather, upholstery, books, and the like, which are injured by great heat, it is, when properly employed, better adapted than any other disinfectant now in use.

Sulphur Dioxide in House Disinfection.—Four pounds of sulphur should be burned for every 1000 cubic feet. The sulphur should be broken into small pieces and put in a pan sufficiently large not to allow the melted sulphur to overflow. This pan is placed in a much larger pan holding a little water. The cracks of the room should be carefully pasted up and the door, after closing, also sealed. Upon the broken sulphur is poured three to four ounces of alcohol and the whole lighted by a match. The alcohol is not only for the purpose of aiding the sulphur to ignite, but also to add moisture to the air. An exposure of eight to twelve hours should be given.

Sulphur fumigation carried out as above indicated is not as efficient as formaldehyde fumigation, but seems to suffice for surface disinfection for diphtheria and the exanthemata. All heavy goods should be removed for steam disinfection if there is any possibility of the infection having penetrated beneath their surface. If there is no place for steam disinfection their surfaces should be thoroughly exposed to fumigation and then to the air and sunlight. In many cases when cleanliness has been observed, surface disinfection of halls, bedding, and furniture may be all that will be required.

There is always a very slight possibility of a deeper penetration of infection than that believed to have occurred; it is, therefore, better to be more thorough than is considered necessary rather than less.

Sulphur dioxide without the addition of moisture has, as already stated under the consideration of disinfectants, very little germicidal value upon dry bacteria.

Public Steam Disinfecting Chambers.—These should be of sufficient size to receive all necessary goods, and may be either cylindrical or rectangular in shape, and are provided with steam-tight doors opening at either end, so that the goods put in at one door may be removed at the other. When large the doors are handled by convenient cranes and drawn tight by drop-forged steel eye-bolts swinging in and out of slots in the door frames. The chambers should be able to withstand a steam pressure of at least one-half an atmosphere, and should be constructed with an inside jacket, either in the form of an inner and outer shell or of a coil of pipes. This jacket is filled with steam during the entire operation, and is so used as to bring the goods in the disinfecting chamber up to the neighborhood of 220° F. before allowing the steam to pass in. This heats the goods, so that the steam does not condense on coming in contact with them. It is an advantage to displace the air in the chamber before throwing in the steam, as hot air has far less

germicidal value than steam of the same temperature. To do this, a vacuum pump is attached to the piping, whereby a vacuum of fifteen inches can be obtained in the chamber. The steam should be thrown into the chamber in large amount, both above and below the goods, and the excess should escape through an opening in the bottom of the chamber, so as to more readily carry off with it any air still remaining. The live steam in the chamber should be under a pressure of two to three pounds, so as to increase its action.

To disinfect the goods, we place them in the chamber, close tight the doors, and turn the steam into the jacket. After about ten minutes, when the goods have become heated, a vacuum of ten to fifteen inches is produced, and then the live steam is thrown in for twenty minutes. The steam is now turned off, a vacuum is again formed, and the chamber again superheated. The goods are now thoroughly disinfected and dry. In order to test the thoroughness of any disinfection, or any new chamber maximum, thermometers are placed, some free in the chamber and others surrounded by the heaviest goods. It will be found that, even under a pressure of three pounds, live steam will require ten minutes to penetrate heavy goods.

The Disinfection of Hands, Instruments, Ligatures, and Dressings for Surgical Operations.

Instruments.—All instruments, except knives, after having been thoroughly cleansed, are boiled for three minutes in a 1 per cent. solution of washing soda. Knives, after having been thoroughly cleansed, are washed in sterile alcohol and wiped with sterile gauze and then put into boiling soda solution for one minute. This will not injure their edges to any great extent.

Gauze.—Gauze is sterilized by moist heat either in an Arnold steam sterilizer for one hour or in an autoclave for thirty minutes. It is placed in a perforated cylinder or wrapped in clean towels before putting in the sterilizer, and only opened at the operation.

Iodoform gauze is best made by sprinkling sterile iodoform on plain gauze sterilized as described above.

Ligatures—Catgut.—Boil for one hour in alcohol under pressure at about 97° C. It is often put in sealed glass tubes, which are boiled under pressure. These remain indefinitely sterile. The alcohol does not injure the catgut. If desired, the catgut can be washed in ether and can be soaked a short time in bichloride before heating in alcohol. Boeckman, of St. Paul, suggested wrapping the separate strands of catgut in paraffin paper and then heating for three hours at 140° C. This procedure prevents the drying out of the moisture and fat from the catgut, so that it remains unshrivelled and flexible after its exposure. Darling, of Boston, tested this method and found it satisfactory. Dry formaldehyde gas does not penetrate sufficiently, and is not reliable. Silver wire, silk, silkworm gut, rubber tubing, and catheters are boiled the same as the instruments.

The Skin of the Patient.—This is washed thoroughly with warm, green soap solution, then with alcohol, and finally with 1:1000 bichloride. A compress wet with a 25 per cent. solution of green soap is now placed on, covered with rubber tissue, and left for three to twelve hours; and after its removal the skin is washed with ether, alcohol, and bichloride solution, and then covered with a gauze compress previously moistened with a 1:1000 bichloride of mercury solution. At the operation the skin is again scrubbed with green soap solution followed by ether, alcohol, and then with the bichloride of mercury solution. In some places the bichloride compress is replaced one hour before the operation by a pad wet in 10 per cent. solution of formalin.

The Hands.—Fürbinger's method, slightly modified, is now much used, and gives good results. The hands are washed in hot soap and water for five minutes, using the nail brush. They are then soaked in alcohol for one minute and scrubbed with a sterile brush. They are finally soaked in a 1:1000 bichloride of mercury solution for three minutes. Another method which gives good results is as follows: Skin of operator is scrubbed for five minutes with green soap and brush, then washed in chloride of lime and carbonate of soda in proportions to make a good lather; washed off in sterile water, and then scrubbed with brush in warm bichloride solution 1:1000.

Sterilized rubber gloves are now being used more and more in operations. The gloves can be sterilized by being left for one minute in boiling 1 per cent. soda solution, or they can be sterilized by steam.

The surgeon's gowns and caps are sterilized by steam. Mucous membranes, as those of the mouth and throat, are cleansed by a solution consisting of equal parts of peroxide of hydrogen and lime-water. In the nostrils it is better to employ the milder solutions, such as diluted Dobell's or Listerine. These are also used in the mouth instead of the peroxide.

The vagina is swabbed out thoroughly with sterile warm soap and water, and then irrigated with a 2 per cent. carbolic acid or a 1:1000 bichloride of mercury solution.

Hypodermic syringes and other syringes when not boiled are sterilized by drawing up into them boiling water a number of times and then finally a 5 per cent. solution of carbolic acid, the acid after three minutes to be washed out by boiling water. If cold water is used the carbolic solution should remain in the barrel for ten minutes. Great care should be taken to wash out all possible organic matter before using the carbolic acid or boiling to sterilize. Syringes made entirely of glass or of glass and asbestos can be boiled in soda solution.

The Sterilization of Milk.

Bacteria when allowed to develop in milk produce fermentation (souring) and render the milk unfit to be used as an article of food, especially for infants. Milk as it reaches the city contains enormous numbers of germs, and these will produce fermentation, even though

the milk be kept on ice. Unclean vessels hasten this process. No matter how good milk may be in the morning, when comparatively fresh, toward evening, unless it has been partly or completely sterilized, it may be dangerous to an infant, and may, especially in summer, cause fatal illness, even though it still tastes sweet.

Complete sterilization destroys all the germs in milk, and so prevents permanently fermentative changes. By partial sterilization most of the germs which are not in the spore form may be destroyed, so that the milk will remain wholesome for at least twenty-four hours in the warmest weather.

Milk is best sterilized by heat, for nearly all chemicals, such as boric acid, salicylic acid, and formalin, are not only slightly deleterious themselves but also make the milk less digestible, and, therefore, less fit for food. It may be sterilized at a high or low temperature—that is, at the boiling temperature—or at a lower degree of heat, obtained by modifying the steaming process.

It has been found that milk sterilized at a high temperature (100° C.) is not desirable for prolonged use, as the high temperature causes certain changes in the milk which make it less suitable as a food for infants. These changes are almost altogether avoided if a temperature below 80° C. is used. It is recommended, therefore, that the lowest temperature be used for partial sterilization which will keep the milk wholesome for twenty-four hours in the warmest weather and kill the tubercle, typhoid, and other non-spore-bearing bacilli. Raising the milk to a temperature of 70° C. for fifteen minutes or 80° C. for twelve minutes will accomplish this. One of the many forms of apparatus is the following:

(*a*) A tin pail or pot, about ten inches deep by nine inches in diameter, provided with the ordinary tin cover which has been perforated with eight holes each an inch in diameter.

(*b*) A wire basket, with eight nursing bottles (as sold for this purpose in the shops).

(*c*) Rubber corks for the bottles and a bristle brush for cleaning them.

Directions (Koplik).—Place the milk, pure or diluted (as the physician may direct), in the nursing bottles and place the latter in the wire basket. Put only sufficient milk for one nursing in each bottle. Do not cork the bottles at first.

Having previously poured about two inches of water in the tin pail or pot and brought it to the boiling point, lower the basket of nursing bottles slowly into the pot. Do not allow the bottles to touch the water or they will crack. Put on the perforated cover and let the steaming continue for ten minutes; then remove the cover and firmly cork each bottle. After replacing the cover, allow the steaming to continue for fifteen minutes. The steam must be allowed to escape freely or the temperature will rise too high.

The process is now completed. Place the basket of bottles in a cool, dark place or in an ice-chest. The bottles must not be opened

until just before the milk is to be used, and then it may be warmed by plunging the bottle in warm water. If properly prepared the milk will taste but little like boiled milk.

The temperature attained under the conditions stated above will not exceed in extreme cases 87° C. (188° F.).

Milk should be sterilized when it is as fresh as possible, and only sufficient milk for twenty-four hours should be sterilized at one time. If after nursing the infant leaves some milk in the bottle this should be thrown away.

Care of the Bottles.—After nursing, the bottles should be filled with a strong solution of washing soda, allowed to stand twenty-four hours, and then carefully cleaned with a bristle (bottle) brush. The rubber corks and nipples should be boiled after using in strong soda solution for fifteen minutes and then rinsed and dried.

After sterilizing milk should never be put into unsterilized bottles, as this will spoil it.

A different but admirable method is the one devised by Dr. Freeman.[1] Here a pail is filled to a certain mark with water, and then placed on the stove until the water boils. It is then removed, and immediately a milk-holder, consisting of a series of zinc cylinders, is lowered with its milk bottles partially full of milk. The cover is again applied. The heat of the outside water raises the temperature of the milk in ten minutes to 75° C. (167° F.), and holds it nearly at that point for some time.[2] After twenty minutes the milk is removed, placed in cold water, and quickly cooled. The milk is kept in the ice-chest until used.

[1] Agent for Pasteurizer, James Dougherty, 411 W. 59th St.

[2] A temperature of 75° C. is advised in Pasteurizing milk, instead of 65° C., which would ordinarily suffice to kill all bacteria free of spores, because of the fact pointed out by Theobald Smith, that the bacteria embedded in the pellicle which forms on the surface are more resistant than those surrounded by fluid.

CHAPTER XI.

THE USE OF ANIMALS FOR DIAGNOSTIC AND TEST PURPOSES.

SUITABLE animals are necessarily employed for many bacteriological purposes. 1. To obtain a development: Thus they may be used as a soil for bacterial growth, when, as in the case of tubercle bacilli, we cannot get a growth in the dead culture media. For this reason material suspected to contain tubercle bacilli is injected into rabbits or guinea-pigs, with the knowledge that, if present, although in too small numbers to be detected by microscopic or culture methods, they will develop in the animals' bodies, and thus reveal themselves. The same may be true of glanders, tetanus, and anthrax bacilli, of pneumococci, of other bacteria, and of protozoa. 2. To cause an increase of one variety of organisms in a mixture: An injection of sputum subcutaneously in rabbits may give rise to a pure pneumococcus septicæmia or a pure tuberculosis. 3. To test virulence: Animals are used to test the virulence or toxin production of organisms, where, as in the case of diphtheria, we have very virulent, attenuated, and non-virulent bacilli of, so far as we know, identical cultural characteristics. Here the injection of a susceptible animal, such as the guinea-pig, is the only way that we can differentiate between those capable of producing diseases from those that are harmless. Still another use of animals is to differentiate between two virulent organisms, which, though entirely different in their specific disease poisons, are yet so closely allied morphologically and in culture characteristics that they cannot always be separated except by studying their action in the animal body both without and under the influence of specific serums upon them. In this way the typhoid and colon bacilli may be separated, or the pneumococcus and streptococcus. 4. To test the antitoxic or bactericidal strength of sera: Diphtheria antitoxin in different amounts is added to one hundred fatal doses of diphtheria toxin and injected into guinea-pigs, and streptococcus immunizing serum is mixed with living streptococci and injected into the vein of a rabbit. 5. To produce antitoxic, bactericidal, or agglutinating sera.

The Inoculation of Animals.—The inoculation of animals may be made either through natural channels or through artificial ones:

1. Cutaneous. Cultures are rubbed into the abraded skin.
2. Subcutaneous. The bacteria are injected by means of a hypodermic needle under the skin, or are introduced by a platinum loop into a pocket made by an incision.
3. Intravenous. The bacteria are injected by means of a hypodermic needle into the vein. This is usually carried out in the ear vein

of the rabbit. If rabbits are placed in a holder, so that the rabbit remains quiet and only the head projects, it is usually easy to pass a small needle directly into one of the ear veins, especially those running along their edges. If the ear is first moistened with a 3 per cent. carbolic acid solution, and then supported between the finger inside and the thumb outside, the vein is usually clearly seen and entered with ease, if a small, sharp needle is held almost parallel with the ear surface and gently pushed into it. When no holder is present, the rabbit can be held by an assistant seizing the forelegs in one hand and the hind in another and holding the rabbit head downward.

4. Into the anterior chamber of the eye.

5. Into the body cavities. The peritoneal and less often the pleural cavities are used for bacterial injection. The hypodermic needle is usually employed, less often a glass tube drawn out to a fine point. The needle or the pointed glass tube is gently pushed through the abdominal wall, moved about to ensure its freedom from the intestines, and the fluid injected.

6. By inhalation. This method is carried out by forcing the animal to inhale an infected spray or dust.

7. By the trachea. This method is carried out by making an incision in the trachea and then inoculating the mucous membrane or injected substances into the trachea and bronchi.

8. Through the intestinal tract by swallowing or the passage of a rubber tube.

9. Into the brain subtance or ventricles after trephining.

In these injections guinea-pigs are held, as a rule, by an assistant grasping in one hand the forelegs and in the other the hindlegs.

Rabbits can be held in the same manner, or, better, placed in some holder or strung up by their hindlegs.

Mice, which are usually inoculated subcutaneously in the body or at the root of the tail, are best placed in a mouse holder, but can be inoculated by grasping the tail in a pair of forceps, and then, while allowing the mouse to hang head downward in a jar, a glass plate is pushed across the top until only space for its tail is left.

All these methods must be carried out with the greatest care as to cleanliness, the hair being clipped and the skin partially, at least, disinfected. The operator must be careful not to infect himself or his surroundings. After the inoculations the animals should be given the best of care, unless, for special purposes, we want to study them under unusual conditions. For food, rabbits and guinea-pigs require only carrots and hay.

If animals die, autopsy should be made at the earliest moment possible, for soon after death some of the species of the bacteria in the intestines are able to penetrate through the intestinal walls and infect the body tissues. If delay is unavoidable, the animals should be placed immediately in a cold place. In making cultures from the dead bodies the greatest care should be taken to avoid contamination. The skin should be disinfected, and any dust prevented by wetting with a 5 per

cent. solution of carbolic acid. All instruments are sterilized by boiling in 3 per cent. soda solution for five minutes. Changes of knives, scissors, and forceps should be made as frequently as the old ones become infected. When organs are examined the portion of the surface through which an incision is to be made must be sterilized, if there is danger that the surrounding cavity is infected, by searing with the flat blade of an iron spatula which has been heated to a dull-red heat. Tissues if removed should be immediately placed under cover after removal so as not to become infected. Sterile deep Petri plates are useful for this purpose.

When it is necessary to transport tissues some distance they should be wrapped in bichloride cloths and sent to the point of destination as soon as possible. In warm weather they may be kept cool by surrounding the vessel which contains them with ice.

Animals rarely show the same gross lesions as man when both suffer from the same infection. The cell changes are similar, and, also, so far as we can test them, the curative or immunizing effects of protective serums.

Leukocytes for Testing Phagocytosis.—Inoculate into the pleural cavity of a rabbit 5 c.c. of a thick suspension of aleuronat powder in a boiled starch solution. The solution should be thick enough to hold the aleuronat in suspension. A 20 to 25 per cent. solution of peptone gives good results. The fluid is withdrawn eighteen to twenty-four hours after the injection.

CHAPTER XII.

THE PROCURING OF MATERIAL FOR BACTERIOLOGICAL EXAMINATION FROM THOSE SUFFERING FROM DISEASE.

A LONG experience has taught me that physicians very frequently take a great amount of trouble, and yet, on account of not carrying out certain simple but necessary precautions, make worthless cultures or send material almost useless for bacteriological study.

In making cultures from diseased tissues various procedures may be carried out, according to the facilities which the physician has and the kind of information that he desires to obtain. From the dead body culture material should be removed at the first moment possible after death. Every hour's delay makes the results less reliable. From both dead and living tissues the less the alteration that occurs in any substance between its removal from the body and its inoculation upon or in culture media or animals the more exact the information which will be obtained from its examination. If the material is allowed to dry many bacteria will be destroyed in the process, and certain forms which were present will be obliterated or, at least, entirely altered in the proportion which they bear to others. If possible, therefore, culture media should be inoculated in the neighborhood of the patient or dead body. For that purpose a bacteriologist should take the most suitable of the culture media to the bedside or autopsy table. Such a list of media, if fairly complete, would comprise nutrient bouillon alone and mixed with one-third its quantity of ascitic fluid, slanted nutrient agar, slanted agar streaked with rabbit or human blood, and firmly solidified slanted blood serum. If only one variety of media is to be used the solidified blood serum is most useful for parasitic bacteria, and this can be easily carried by the physician and inoculated by him, even if he is not very familiar with bacteriological technique. The material must be obtained in different ways, according to the nature of the infection.

For the detection of the bacteria causing septicæmia we are met with the difficulty that there are apt to be very few organisms present in the blood until shortly before death. It will, therefore, be useless to take only a drop of blood for cultures, as even when present there may not be more than eight or ten organisms in a cubic centimetre. If cultures are to be made at all, it is, therefore, best to make them correctly by taking from 5 to 20 c.c. of blood by means of a sterile hypodermic needle, or a suitable glass tube armed with a hypodermic needle, from the vein of the arm, after proper cleansing of the skin and a tiny incision. Into each of five different tubes containing bouillon we add 1 c.c. of blood, and into a flask containing 100 c.c. we

add 5 c.c. We have made by this mixture of blood and bouillon a most suitable medium for the growth of all bacteria which produce septicæmia, and at the same time have added a sufficient quantity of blood to ensure us the best possible chance of having added some of the bacteria producing the disease. We also add to each several tubes of melted nutrient agar, at 40° C., 1 c.c. of blood and pour into Petri plates, so as to indicate roughly the number of organisms present if they happen to be in abundance. From wounds, abscesses, cellulitis, etc., the substance for bacteriological examination can, as a rule, best be obtained by means of a syringe, or when opened, by small rods armed with a little absorbent cotton. A number of these can be carried in a test-tube. Both rods and tubes must be sterile. The swab is inserted in the wound, then streaked gently over the oblique surface of the nutrient agar in one tube, over the blood serum in another, and then inserted in the bouillon. Finally, either at the bedside or in the laboratory, material is thinly streaked over the surface of nutrient agar contained in several Petri dishes. We inoculate several varieties of media, with the hope that one at least will prove a suitable soil for the growth of the organisms present. From surface infections of mucous membranes, as in the nose, throat, vagina, etc., the swab, again, is probably the most useful instrument for obtaining the material for examination. The greatest care, of course, must be used in all cases to remove the material for study without contaminating it in any way by other material which does not belong to it. Thus, for instance, if we wish to obtain material from an abscess of the liver, where the organ lies in a peritoneal cavity infected with bacteria, here one must first absolutely sterilize the surface of the liver by pressing on it the blade of a hot iron spatula before cutting into the abscess, so that we may not attribute the infection which caused the abscess to the germs which we obtained from the infected surface of the liver. From such an organ as the uterus it is only with the greatest care that we can avoid outside contamination, and only an expert bacteriologist familiar with such material will be able to eliminate the vaginal from the uterine bacteria.

A statement of the conditions under which materials are obtained should always accompany them when sent to the laboratory for examination, even if the examination is to be made by the one who made the cultures. These facts should be noted, or otherwise at some future date they may be forgotten and misleading information sent out. The work of obtaining material for examination without contamination is at times one of extreme difficulty. It simply must be remembered that if contamination does take place our results may become entirely vitiated, and if the difficulties are so great that we cannot avoid it, it may simply mean that under such conditions no suitable examination can be made. Where the substance to be studied cannot be immediately subjected to cultures or animal inoculations it should be transferred in a sterile bottle as soon as possible to a location where the cultures can be made. If for any reason delay must take place, the

material should at least be put in a refrigerator, where cold will both prevent any further growth of some varieties of bacteria and lessen the danger of the death of others. After having made the cultures, some of the infected material should always be smeared on a couple of clean slides or cover-glasses and allowed to dry. These can be stained and examined later, and may give much valuable information.

In obtaining samples of fluid, such as urine, feces, etc., the bottles in which they are placed should always be sterile, and, of course, no antiseptic should be added. It is necessary to clearly explain this to the nurse, for she has probably been instructed to add disinfectants to all discharges. Disinfected material is, of course, entirely useless for bacteriological investigations. It cannot be too much emphasized that materials which are not immediately used should be sent to the laboratory as quickly as possible, for in such substances as feces, where enormous numbers of various kinds of bacteria are present, those which we seek most, such as the typhoid bacilli, frequently succumb to the deleterious products of the other bacteria present. Even when abundantly present living typhoid bacilli may entirely disappear from the feces in the course of even twelve hours, while at other times they may remain present for weeks. These differences depend on the associated organisms present, the chemical constitution of the feces or urine, and the conditions under which the material is obtained.

Not only for obtaining fluid for agglutination purposes, but also for examination for peculiar bodies in the exanthemata, blister fluid is valuable. A blister can be raised quickly by placing a little strong ammonia on the skin and covering with a watch-glass, or more slowly by a cantharides plaster.

CHAPTER XIII.

THE RELATION OF BACTERIA TO DISEASE.

In preceding chapters we have considered the chemical effects of bacteria and their ferments on dead organic substances. Now we have to consider the growth of bacteria in the living host and the results of such development. While it is true that there is a great difference between living and dead matter, and that, therefore, the living animal cannot be looked upon as merely a quantity of peculiarly specialized material to be used for food for bacterial growth, still, in a very real sense, we are warranted in considering the infected living body as a food mass subject to bacterial growth. The difference is that besides the chemical substances, temperature, and conditions inherent to the fluids of the living body and its tissues, micro-organisms have also to reckon with the constant production of new substances by the living cells of the invaded organism, which may be antagonistic to them. In the production of lesions by micro-organisms there are four main factors involved—viz., on the part of micro-organisms, the power to elaborate poison and the ability to multiply; on the part of the body, the degree of sensitiveness to the poisons of the bacteria and the tendency to produce antitoxic or bactericidal substances. No known variety of bacterial cell has as a single organism the ability to produce enough poison to do appreciable injury in the body, nor is it probable that there is any variety which, if it multiplied in the body to the extent that some pathogenic bacteria are capable of, would not produce disease. As already mentioned, varieties of bacteria even under similar conditions differ enormously in the amount of poison which they produce and in their ability after gaining entrance to multiply in the body.

To understand the bacterial factor in the production of disease we must recognize that both the body invaded and the bacteria which invade are living organisms, and that the products of the cellular activity of the body act on the bacteria at the same time the bacterial products act upon the human body. Just as there are different races and species of animals having dissimilar characteristics, there are different races and species among bacteria, and just as the descendants of one animal species under changing conditions gradually become diverse, so do the descendants of one bacterial species. In fact, the rapidity of the development of new generations of bacteria allow in them of much quicker changes under new conditions than are possible in the higher animals and plants. Considering these and other facts, we can readily understand how the different types of bacteria do not grow equally well in every variety of animal, and after discovering that there are variations

in the bactericidal properties of the blood from day to day we are not surprised that they do not find the body of the same animal always equally suitable. The study of bacteria in the more simple and known conditions of artificial culture media has shown us how extremely sensitive many bacteria are to slight chemical, and other changes. In media conditions favorable to growth may still be unfavorable for toxin production.

If we take specimens of diphtheria bacilli from three different cases of diphtheria, we sometimes find that on growing them for several days in suitable bouillon one will have produced poison in the culture fluid to such a degree that a single drop suffices to kill a large guinea-pig; the second, grown in a similar manner, will kill another animal of the same size with half a drop; while the third will kill with one-tenth of a drop. This illustrates the important fact that different varieties of the same bacillus have different toxin-producing powers under the same conditions—that is, that the conditions that are suitable for the full development of the functions of one strain are not so for another strain.

Let us now cultivate these same strains in bouillon which is a little too acid or a little too alkaline for their maximum development, and we shall find that while all of them will grow, only one and probably that one which produced the most toxin under favorable conditions will continue to develop it, while the others will fail to produce any specific poison. This illustration makes clear one reason for the variation in severity among different cases in an epidemic, since the conditions in one throat may favor growth but not toxin production, while in another throat both are favored. The fact that growth of bacteria may occur in the body and yet no specific poison be produced, and that, of the same species of bacteria, some varieties are capable of producing toxin under less favorable circumstances than others, is very important to remember.

The cultivation of the tetanus bacillus also furnishes some interesting facts which illustrate the complicated ways in which the growth of varieties of bacteria are hindered or assisted. The tetanus bacillus, when placed in suitable media, will not grow except in the absence of oxygen; but place it under the same conditions, together with a micro-organism which actively assimilates oxygen, and the two in association will grow in the presence of air. As a rule, when tetanus bacilli are driven into the flesh by a dirty nail or blank cartridge plug, aërobic bacteria are driven in also and so help to further infection.

The influenza bacillus is a striking example of the special requirements of certain bacteria. On culture media it will thrive only in the presence of hæmoglobin.

It is evident, therefore, that for each variety of organism there are special conditions requisite for growth, and that a temperature, degree of acidity, kind of food, supply of oxygen, etc., suitable for one may be utterly unsuitable for another; that, still further, when two organisms grow together one may so alter some of these conditions as to render unsuitable ones suitable, and *vice versa*.

Let us now consider some of the facts which have been observed concerning the growth of bacteria in the living body as contrasted with culture media. In the first place, it has been learned, as will be described in the latter part of the book, that each variety of bacteria can incite only certain types of infection. Indeed, because of this fact, the majority of bacteria which excite disease can be traced back for thousands of years by means of the records, these parasitic bacteria breeding true and keeping distinct from the great mass of bacteria occurring in the air, water, and soil.

Parasitic bacteria have gradually adapted themselves not only to certain species of animals, but to certain circumscribed areas of the body. Thus the diphtheria bacilli grow chiefly upon the mucous membranes of the respiratory tract, but cannot develop in the blood or in the subcutaneous tissues. The cholera spirilla develop in the inflamed intestinal mucous membrane, but cannot grow in the respiratory tract, blood, or tissues. The tetanus bacilli develop in wounds of the subcutaneous tissues, but cannot grow on the intestinal mucous membranes or in the blood.

Other bacteria find, indeed, certain regions especially suitable for their growth, but under conditions favorable for them are capable of developing in other locations. Thus, the typhoid bacillus grows most luxuriantly in the Peyer patches and mesenteric glands, but also invades the blood, spleen, and other regions. The tubercle bacillus often remains localized in the apex of a lung or a gland for years, but at any time may invade many tissues of the body. The gonococcus finds the mucous membrane of the genitourinary tract most suitable for its development, but also frequently is capable of growth in the peritoneum and even sometimes in the general circulation. The pneumococcus develops most readily in the lungs, but also invades the connective tissues, serous membranes, and the blood.

All these bacteria, although ordinarily increasing only in the body of man, can be grown on suitable dead material.

There are bacteria which, in so far as we know, find the bodies of human beings or animals the only fit soil for their growth. These are strictly the true parasites. The spirillum of relapsing fever grows only in man; neither the food nor the conditions suitable for the development of this micro-organism outside of the body have as yet been discovered.

Following rather closely the schematic separation of bacteria according to their relation to disease we might classify them as:

1. *Strict saprophytes*, or bacteria which grow readily in suitable dead organic material, but not in the body under ordinary conditions.

a. Bacteria which in their growth produce no substances poisonous to the body, which are capable of absorption through the intestinal walls or act on its epithelium.

b. Bacteria which produce in their growth in dead organic matter poisons capable of acting on the mucous membrane or of being absorbed into the animal body.

2. *Parasites*, with possibility of saprophytic growth. These are bacteria which can develop either as parasites or saprophytes. The different varieties vary as to the amount of poison which they produce. Some grow luxuriantly in dead organic material under very diverse conditions, others only under specially favorable conditions. In the body they also vary—some grow extensively in the blood, while others are limited to one or more tissues, some being widely disseminated throughout the body, while others are localized in or upon a certain portion of it.

3. *Strict parasites*, or bacteria which, so far as we know, grow only in the living animal or vegetable organism. These again vary in the amount of poison which they produce and in the local or general infection to which they give rise.

Adaptation of Bacteria to the Soil upon which They Are Grown.—Those bacteria which grow both in living and dead substances vary from time to time as to their readiness to develop in either the one or the other. As a general rule, bacteria grown in any one medium become more and more accustomed to that and other media more or less analogous to it, while, on the other hand, they are less easily cultivated on media widely different from that in which they have developed. Thus we have a culture of tubercle bacilli which, after having grown for three years in the bodies of guinea-pigs, will no longer develop on dead organic matter, while a bacillus which was obtained from the same stock, but grown on bouillon for three years, will no longer increase in the animal body. From the same stock, therefore, two varieties have developed, the one being now practically a saprophyte and the other a parasite.

Local Effects Produced by Bacteria and Their Products.—After the bacteria gains entrance to a suitable part of the body and find conditions favorable for growth there is a certain lapse of time before sufficient bacterial poisons have accumulated to cause by their action on the tissue noticeable disturbance. This is called the period of incubation. Its length depends on the amount, kind, and virulence of the micro-organisms introduced and the tissue invaded. The incubation period over, we note the course of the local and general lesions excited by the specific and general poisons. The extent to which this will progress depends, on the one hand, on the characteristics of the invading micro-organisms; on the other, on the characteristics of the tissues invaded.

The local effects of the bacterial poisons upon the cells give rise to the various kinds of inflammation, such as serous, fibrinous, purulent, croupous, hemorrhagic, necrotic, and gangrenous, and also proliferative, as seen, for example, in leprosy. Some bacteria incite specific forms of inflammation along with those common to many bacteria; others produce no peculiar form of lesions.

Thus inflammation and serous exudation into the subcutaneous tissues follow injections of the pneumococcus or anthrax bacillus. The development of the streptococcus or pneumococcus in the endocardium or pleural cavity is followed by a serous exudation, frequently with more or less fibrin production. The formation of pus results more

especially from the streptococcus, pneumococcus, and staphylococcus; but nearly all forms of bacteria, when they accumulate in one locality, may produce purulent inflammation. The colon, typhoid, and influenza bacilli frequently cause the formation of abscesses.

Catarrhal inflammation, with or without pus, follows the absorption of the products of many bacteria, such as the gonococcus, pneumococcus, streptococcus, and influenza bacillus, etc. The hemorrhagic exudation seen in pneumonia is usually due to the pneumococcus; it is observed also in other infections. Cell necrosis is produced frequently by the products of the diphtheria and of the typhoid bacilli and by those of other bacteria. Specific proliferative inflammation follows the localization of the products derived from the tubercle bacillus and the leprosy bacillus.

Not only can the poisons of one species of bacteria, according to the tissues attacked, produce several forms of inflammation, but the same organism will vary as to its mode and extent of invasion; this depending, first, upon its own characteristics at the time as to virulence, etc., and, second, upon the conditions in the infected animal, such as its health and power of resistance, the location of infection, and the circumstances under which the animal remains. Such variations, therefore, are in no case specific, for different poisons will produce changes which appear identical.

Manner in which Bacteria Produce Injury.—The actual mechanical presence of the bacteria is only of importance when, as in pronounced septicæmia or pyæmia, they exist in such enormous numbers as to interfere mechanically with the circulation or cause minute thrombi, and later emboli, which finally produce infarction and abscesses in different parts of the body. Even these dangerous effects are almost wholly due to the chemical substances given off, which are more or less directly poisonous. Some portion of the protoplasm of almost every variety of bacteria acts as an irritant to tissues and combines with some of the body cells, and that of most have a positive chemotaxis.

These poisonous products, as already described in the previous chapter, can be separated from the culture fluid in which the bacteria have grown or they can be extracted from their bodies. These products without the bacteria themselves injected into animals cause essentially the same lesions as are produced by the bacteria when they develop in the animal body. The substances contained in or produced by the bacteria, with few exceptions, attract the leukocytes, and when great masses of bacteria die suppuration usually follows.

General Symptoms Caused by Bacterial Poisons Absorbed into the Circulation.—Fever is produced under favorable conditions by all bacterial poisons. The first requisite is that sufficient poisons be absorbed; but, on the other hand, they must not be absorbed with such rapidity as to overwhelm the infected, for a moderate dose may raise the temperature, while a very large dose lowers it, as occurs sometimes when a very large surface, such as the peritoneum, is suddenly involved. The effect of fever has no known antibacterial power, but it may be due to

some part of the reaction of the tissues which in other portions gives rise to the antitoxins and antibactericidal substances. It is also a sign that the body cells as a whole are not yet overwhelmed by the infection.

With few exceptions the bacterial poisons produce an increase in the number of leukocytes and a lessening in the amount of hæmoglobin in the blood. In uncomplicated infection with typhoid bacilli there is a hypoleukocytosis. The different varieties of leukocytes are increased in varying proportions in different infections. The red-blood cells are directly injured by a number of bacterial substances. The deleterious effects on the nutrition are partly due to the direct effect of the poison and partly to the diseased conditions of the organs of the body, such as the spleen, kidney, and liver. Degeneration of the nerve cells is frequently noticed after infectious diseases; especially is this true of diphtheria. Several bacterial poisons have been found to produce convulsions; the best example of this is the tetanus toxin.

Influence of Quantity in Infection.—With pathogenic bacteria the number introduced has an immense influence upon the probability of infection taking place.

If we introduce into a culture medium containing some fresh human blood orserum a few bacteria it is probable that they will all die; whereas if a greater number are introduced, while there will at first be a great diminution of these, those that die, having combined with the bactericidal substances in the serum, neutralize them; then those bacteria which survive begin to increase, and soon they multiply enormously. The same is true for parasitic bacteria in the body. A few only gaining entrance, they may die; a larger number being introduced, some may or may not survive; but if a still greater quantity is injected it is almost certain that there will be some surviving members, which, because of the antagonistic substances, having been neutralized or of their having some peculiar properties, will begin to grow and excite disease.

With those bacteria whose virulence is great a very few organisms will produce disease almost as quickly as a million, allowance only being made for the short time required for the few to become equal in number to the million. At the other extreme of virulence, however, many millions may have to be introduced to permit of the development of any of the organisms in the body. With these bacteria we are thus able to produce either no effect whatever, a local effect, or in some cases a general septicæmia, by regulating the amount of infection introduced. In the majority of cases in man the number of bacteria received is comparatively small; but by the rupture of an abscess into a body cavity or into the circulation, or by the opening of the intestinal contents into the peritoneum, the quantity introduced may be enormous.

Variation in Degree of Virulence Possessed by Bacteria.—Bacteria differ, as has already been stated, as to the ease and rapidity with which they grow in any nutritive substance and the amount of poison they produce. Both of these properties not only vary greatly in different members of the same species, but each variety of bacteria

may to a large extent be increased or diminished in virulence. The septicæmic class of bacteria when grown in the body fluids gradually produce cells with less substance with affinity for the bactericidal substances of the blood and thus become less vulnerable. The variation in the amount of specific poisons produced by bacteria can be best studied in diphtheria and tetanus. We note, first, that different cultures of diphtheria and tetanus bacilli have wide variations in the amount of toxin which they produce—*i. e.*, a diphtheria bacillus obtained from a case of diphtheria will produce in suitable nutrient broth a poison of such strength that 1 c.c. will kill an average-sized guinea-pig, while the poison from another bacillus will kill with a much less quantity, or 0.005 c.c. Further, the bacilli obtained from some sources retain their power of producing poison, when grown on artificial media, for years unaltered, while others lose much of this in a few months. This is equally true of the tetanus bacilli.

The power to produce toxin can be taken from bacilli by growing them under adverse circumstances, such as cultivation at the maximum temperature at which they are capable of development. Some bacilli are easily attenuated; others are robbed of their virulence only with great difficulty. Increase of toxin-production is more difficult, and it is only possible to obtain it to a certain extent. The means usually employed are the frequent replanting of cultures But with all our efforts we are usually only able to restore approximately the degree of toxin-formation which the cultures originally possessed. The adaptation of bacteria to any nutritive substance, living or dead, so that they will grow more readily, is more easily brought about, provided they will grow at all. The streptococcus from erysipelas and the pneumococcus from pneumonia are typical of this class of bacteria. Inoculate a rabbit with a few streptococci obtained from a case of human sepsis, and, as a rule, no result follows; inject a few million, and usually a local induration or abscess appears; but if one hundred million are administered septicæmia develops. From this rabbit now inoculate another, and we find that a dose slightly smaller suffices to produce the same effect; in the next animal inoculated from this still less is required, and so on, until in time, with some cultures, a very minute number will surely develop and produce death. The same increase in virulence can be noted when septic infection is carried in surgery or obstetrics from one human case to another. By allowing bacteria to continue to develop under certain fixed conditions they become accustomed to them, and less adapted for all that differ.

Somewhat distinct, again, from that class of bacteria which multiply rapidly are those which, like the tubercle and leprosy bacilli, because of not developing in the blood increase more slowly. Here increase of virulence is shown, as before, by the production of disease through the introduction of very small numbers into the body, but increase in rapidity of development cannot progress except to within certain limits. A single streptococcus may, through its rapid multiplication, produce death in eighteen hours; a single tubercle bacillus, on the

other hand, cannot produce sufficient numbers in less than two weeks. The virulence of the septicæmic class of bacteria is not at all the same when measured in different animals, and it is largely for this reason that the virulence in test animals does not usually correspond with the severity of the case from which the organism was derived. We should remember in this connection the varying power of resistance in different animals to the same organism and of the same individual at different times.

Mixed Infection.—The combined effects upon the tissues of the products of two or more varieties of pathogenic bacteria, and also of the influence of these different forms on each other, are of great importance in the production of disease. The infection from several different organisms may occur at the same time, or one may follow the other or others—so-called secondary infection. Thus, an abscess is often due to several forms of pyogenic cocci. If a fresh wound is infected from such a source the inflammation produced will probably be caused by all the varieties present in the original infection. Peritonitis following intestinal injuries must necessarily be due to more than one organism. Thus, whenever two or more varieties of bacteria are transferred to a new soil, mixed infection takes place if more than one variety is capable of developing in that locality.

Forms of infection which are allied to both mixed and secondary infection are those occurring in the mucous membranes of the respiratory and digestive tract. In these situations pathogenic bacteria of slight virulence are always present even in health. Thus, in the upper air passages there are usually found streptococci, staphylococci, and pneumococci. When through a cold, or the invasion of another infective agent, as the diphtheria bacillus, the virus of smallpox or scarlet fever, the epithelium of the mucous membrane of the throat is injured or destroyed, the pyogenic cocci already present are now enabled in this diseased membrane to grow, produce their poison, and even invade deeper tissues. The intestinal mucous membrane is invaded in a similar way by the colon bacilli and other organisms after injury by the typhoid or dysentery bacilli or cholera spirilla. Generally speaking all inflammations of the mucous membranes and skin contain some of the elements of mixed infection. Blood infection, on the other hand, is usually due to one form of bacteria, as even when several varieties are introduced, only one, as a rule, is capable of development. The same is true to a somewhat less extent of inflammation of the connective tissue. The additional poison given off by the associated bacteria aid infection by the primary invaders by causing a lowering of the vital resistance of the body. In some cases the secondary infection is a greater danger than the primary one, as pneumococcic bronchopneumonia in laryngeal diphtheria or streptococcic septicæmia in scarlet fever and smallpox.

The bacteria are also at times directly influenced by the products of associated organisms. These may affect them injuriously, as, for example, the pyogenic cocci in anthrax; or they may be necessary to

their development, as in the case of anaërobic bacteria. Not infrequently the tetanus bacilli or spores would not be able to develop in wounds were it not for the presence of aërobic bacteria introduced with them. This is shown outside the body, where tetanus bacilli will not grow in the presence of oxygen unless aërobic bacteria are associated with them. Again, it is found that the association of one variety with another may increase its virulence. Streptococci are stated to increase the virulence of diphtheria bacilli, but here it is probably the loss of resistance of the tissues because of the streptococcic poison. On the other hand, the absorption of the products of certain bacteria immunizes the body against the invasion of other bacteria, as shown by Pasteur that attenuated chicken-cholera cultures produce immunity against anthrax.

Ability of Bacteria to Penetrate the Skin and Mucous Membranes. THE SKIN.—The unbroken skin is a great protection against the penetration of micro-organisms. When they do penetrate it is through the glands, or more often through some unobserved wound. Soluble vegetable poisons, such as aconite and bacterial toxins, are not absorbed.

There is an apparent exception to the above statements in the fact that the pyogenic staphylococci and sometimes the streptococci exist upon the skin or in it between its superficial horny cells, some exceptional circumstances, such as wounds or burns, being required to allow the organisms to penetrate deeper. The cutaneous sweat glands, and the hair follicles with their appended sebaceous glands, may allow entrance of infection, as various incidents may lead to the introduction and retention of virulent micro-organisms. When this occurs the retained products may lead to necrosis of the epithelium and thus allow the bacteria to penetrate to the deeper tissues. The secretion of the sebaceous glands appears not to be bactericidal, but the acidity of the perspiration renders it slightly so.

SUBCUTANEOUS CONNECTIVE TISSUES.—Many bacteria cannot develop in the connective tissues and others produce a milder infection there than elsewhere. Others develop readily and cause infection. The rapidity of development of new connective tissue and the bactericidal properties of the lymph are the main known hindrances to infection.

THE MUCOUS MEMBRANES.—The moist condition of the surface of the membranes aids bacterial multiplication. Mucus is only slightly bactericidal for some bacteria and not at all for others. Bacteria, such as the pneumococci and streptococci, remaining in it become somewhat attenuated. The conjunctival mucous membranes are protected by the cleansing produced by the flow of the lacrymal secretion and by its slight germicidal action. In infancy the membranes are readily infected by gonococci and later by pneumococci, the Koch-Weeks bacillus and others. Many soluble poisons and toxins are absorbed. The mucous membranes of the nasal cavity are somewhat cleansed by the nasal secretion, which is feebly bactericidal. The deeper portions of the nasal cavity are usually the seat of streptococci and other bacteria. The mouth in a person in health is cleansed by the feebly

bactericidal saliva. When the teeth are decayed many varieties of bacteria abound. The bacteria, such as the diphtheria bacilli, streptococci, etc., rarely invade the mucous membrane of the tongue or mouth.

The tonsils with their crypts are usually the seat of the pyogenic cocci and are readily infected by the diphtheria bacilli and others. Whether the absolutely intact epithelium allows the passage of these bacteria is disputed, but with the slight pathological lesions usually present it undoubtedly does.

THE LUNGS.—Most inhaled bacteria which pass the larynx are caught in the bronchi. Many of these are gradually removed by the ciliated epithelium. Both the alveolar epithelial cells and the leukocytes which enter the air sacs and bronchioles have been shown to take up bacteria. The normal lung is, therefore, rapidly freed of bacteria. Under the influences of certain nervous impressions such as follow exposure to cold, etc., certain areas of the lung seem to lose their protective defences.

THE STOMACH.—The pure gastric juice, through the hydrochloric acid it contains, is able to kill most non-spore-bearing organisms in a short time, but because of neutralization through food, or because the bacteria are protected in the food, many of them pass into the intestines. Tubercle, typhoid, colon, and dysentery bacilli, when fed by the mouth with food, readily pass through the stomach. Certain acidiphilic germs, as well as yeasts and torulæ, seem to grow in the gastric secretion; these are largely non-pathogenic. Perforation of the stomach is usually followed by peritonitis, because of the irritant effect of the gastric juice and the presence of bacteria which are temporarily retained. The gastric juice neutralizes tetanus and diphtheria toxins. Other poisons, such as some that occur in decayed meat, are not neutralized. The stomach is exceptionally free from bacterial inflammations.

INTESTINES.—The bile is feebly bactericidal for some bacteria, but, on the whole, the intestinal secretions have little or no germicidal power. The number of bacteria increase steadily from the duodenum to the head of the colon, and diminish slightly from the upper to the lower end of the colon. The pancreatic juice destroys many of the toxic bacterial products. The presence of the bacilli of the colon group, of streptococci, etc., do not often set up any inflammatory condition in the normal intestines of healthy persons. In children suffering from the prostrating effects of heat they are apt to excite inflammatory changes. Even pathogenic bacteria, such as the typhoid, dysentery, and tubercle bacilli, may pass through the whole length of the healthy intestines without inciting inflammations. Slight lesions aid the passage of bacteria to the deeper structures.

Importance of Location of Point of Entry of Bacteria. Most bacteria cause infection only when they gain access to special tissues and must, therefore, enter through certain portals. This fact is of immense importance in the transmission or prevention of disease. Thus, for example, let us rub very virulent streptococci, typhoid bacilli, and diphtheria bacilli into an abrasion on the hand. The typhoid bacillus produces no

lesion, the diphtheria bacillus but a very minute infected area, but the streptococcus may give rise to a severe cellulitis or fatal septicæmia. Now place the same bacteria on an abrasion in the throat. The typhoid bacillus is again harmless; the diphtheria bacillus produces inflammation, a pseudomembrane, and toxæmia, and the streptococcus causes an exudate, an abscess, or a septicæmia. Finally, introduce the same bacteria into the intestines, and now it is the typhoid bacillus which produces its characteristic lesions, while the streptococcus and diphtheria bacillus are usually innocuous.

If we tried in this way all the parasitic bacteria we would find that certain varieties are capable of developing and thereby exciting disease only on the mucous membrane of the throat, others of the intestine, others of the urethra; some develop only in the connective tissues or in the blood, while others, again, under favorable conditions, seem able to grow in or upon most regions of the body.

The Dissemination of Disease.—The spread of infection is influenced by: 1. The number of species of animals subject to infection.

Many human infectious diseases do not occur in animals, and many animal infections are not found in man. Thus, so far as we know, gonorrhœa, syphilis, measles, smallpox, typhoid fever, etc., do not occur in animals under ordinary conditions; while tuberculosis, anthrax, glanders, hydrophobia, and some other diseases are common to both man and animals.

2. The quantity of the infectious material and the manner in which it is thrown off from the body.

In diphtheria, typhoid fever, cholera, pulmonary tuberculosis, septic endometritis, influenza, and gonorrhœa enormous numbers of infectious bacteria are cast off through the discharges from the mouth, intestines, and genitourinary secretions, causing great danger of infection. On the other hand, in tuberculous peritonitis, relapsing fever, septic pleurisy and endocarditis, gonorrhœal rheumatism, and the like, there is little or no danger of infecting others, as few or no bacteria are cast off.

3. The resistance of the infectious bacteria to the deleterious effects of drying, light, heat, etc.

In this case the presence or absence of spores is of the greatest importance. The spore-bearing bacilli, such as tetanus, anthrax, etc., being able to withstand destruction for a long time, retain their power of producing infection for months or even years after elimination from the body. The bacteria which form no spores show great variation in their resistance to outside influences. Some of these, such as the influenza bacilli and the gonococci, the virus of syphilis and hydrophobia, are extremely sensitive; the pneumococci, cholera spirilla, glanders bacilli, etc., are a little hardier; then follow the diphtheria bacilli, and after them the typhoid and tubercle bacilli and the staphylococci.

4. The ability or the lack of ability to grow outside of the infected tissues.

Such bacteria as the pneumococcus, tubercle, influenza, diphtheria, glanders, and leprosy bacilli do not develop, as far as we know, outside of the body under ordinary conditions. Under exceptional circumstances, as in milk, some may develop. Others, again, such as the streptococcus and staphylococcus, typhoid and anthrax bacillus, the cholera spirillum, and some anaërobics, may develop under peculiar conditions existing in water or soil.

While for the pathogenic bacteria, as a rule, the saprophytes met with in the soil and water are antagonistic, yet in some cases—and especially is this true of the anaërobic bacteria—they are helpful. Such bacilli as tetanus are believed to require the association of anaërobic bacteria to permit of their development in the soil in the presence of oxygen.

5. Ability to develop in or upon some portion of the skin or mucous membrane, either after or before disease, and without causing infection. As complete a knowledge as possible of this saprophytic development in man of parasitic bacteria is necessary if we are to combat the spread of infection. In the superficial layers of the epithelium and on the surface of the skin we find the different pyogenic cocci, which are capable of infecting a wounded or injured part or causing inflammation in the glands. Acne, the pustules in smallpox, the pus on a burned surface, boils, etc., all come from these pyogenic cocci. In surgical cases the skin has to be as thoroughly disinfected as possible, to prevent the formation of stitch-hole abscesses and wound suppuration.

In the secretion of the mucous membrane covering the pharynx and nasopharynx there is always an abundance of bacteria. In throats examined in New York City, streptococci, staphylococci, and pneumococci are found in almost every instance, and even in the country they are often present. In the anterior nares there are fewer parasitic bacteria than in the posterior portions. The nasal secretion is only very slightly, if at all, bactericidal. Many other varieties of bacteria, such as the meningococci and the influenza bacilli, are probably often present in small numbers. In those constantly in contact with cases of diphtheria, and in those convalescent from diphtheria, virulent diphtheria bacilli are frequently found in the throat.

After exposure to cold or injury of any kind, owing to the presence of these bacteria, the persons harboring them may develop tonsillitis, tonsillar abscess, or diphtheria; or the bacteria may invade the bronchial mucous membrane or the lungs. The diphtheria bacilli, and perhaps other bacteria, transmitted to others may become the source of infection to them, though the person who spreads the infection may remain unaffected.

After typhoid fever the bacilli may remain in the intestinal contents for weeks and in the bladder and gall-bladder for months.

Autoinfection.—Some good observers have stated that bacteria can be absorbed through the intestinal wall into the chyle and blood. When the intestinal canal is injured, or its circulation hindered by strangulation, etc., the bacillus coli and some other bacteria may

penetrate through the injured walls and cause peritonitis or general infection. Under certain conditions, as during the debility due to hot weather, the bacteria in the intestines cause, through their products, irritation, and in children even serious intestinal inflammation.

The kidneys, bladder, and urethra may be the source of infection and may give rise to disease. Long after an acute gonorrhœa has passed gonococci may remain in sufficient numbers to cause a new inflammation or produce infection in others. A cystitis may run on chronically for years, and then suddenly become acute or spread infection to the kidneys. A persistent gonorrhœal vaginal infection may lead to a gonorrhœal endometritis, or salpingitis, or peritonitis, under suitable conditions. The staphylococci in the skin and the colon bacilli and pyogenic cocci in the fecal discharges may also be carried into the bladder and uterus and produce septic infection.

Occasionally the casting off of the bacteria allows them to infect other places, as in some cases where laryngeal and intestinal tuberculosis follow pulmonary tuberculosis. We must bear in mind, however, that infection in these regions may have been produced through the lymph- and blood-channels.

In nearly all cases of infection the products of bacterial growth are absorbed into the blood, and along with them a few bacteria also, even when they do not reproduce themselves in it. The greater the extent of the infection and the more deep seated it is, the greater is the amount of absorption. The bacteria enter the blood, according to Kruse, by (1) passive entrance through the stromata of the capillary walls; (2) carriage into the blood in the bodies of leukocytes; (3) growth of the bacteria through the walls of the vessels; (4) transmission of the bacteria through the lymph glands placed between the lymph- and blood-vessels.

When bacteria are abundant in the blood they become fixed in the capillaries of one or all of the organs, especilly of the liver, kidneys, spleen, and lungs, and then, by means of the leukocytes, which penetrate the capillary walls, or, directly, they pass into the tissues and substance of the organs. They thus reach the lymph channels and glands, or through the secretions gain entrance into the gall-bladder, saliva, etc., or press through the epithelium, as in the alveoli of the lungs; more rarely they pass through the excretions into the urine, as in typhoid fever, though some deny that this can happen unless there is a previous inflammation of the kidneys.

Elimination of Bacteria through the Milk.—The passage of bacteria through the breast is important, from the fact that milk is so largely used as food. Many observers have reported the finding of tubercle bacilli in milk when the gland itself was intact and the animal tuberculous. Some authorities have put its presence in milk, under these circumstances, as high as 50 per cent. of the cases. This, in our experience, is undoubtedly too high, and probably these observers have been deceived by the pseudotubercle bacilli. They are undoubtedly present, however, in the milk of some animals in which tuberculous disease of

the gland could not be demonstrated. The finding of streptococci and staphylococci is due probably in the majority of cases to the infections taking place as the milk is voided, for the epithelium at the outlet of the lacteal ducts is always infected with staphylococci, and usually streptococci, which have often been received from the mouth of the sucking infant.

Elimination of Bacteria by the Skin and Mucous Membranes.—Whether bacteria pass from the blood by the sweat is a mooted point. The skin is always the seat of the staphylococcus and frequently of other bacteria, so that it is difficult to determine in any given case the origin of the bacteria found in the sweat. Many observers have reported the passage of bacteria from the blood through the mucous membrane. So long as the organs of secretion are not injured it is not likely that many micro-organisms are eliminated from the blood in this way. Bacteria are sometimes eliminated through the urine, but here, as a rule, when great numbers of organisms are found, it is due to development in the bladder. Such removal, moreover, has little if any beneficial effect; but, on the other hand, it may be a source of danger to others, as in typhoid fever. The removal of the poisonous products of bacteria by the kindeys, intestines, etc., on the contrary, is of great advantage to the organism.

CHAPTER XIV.

THE ANTAGONISM EXISTING BETWEEN THE LIVING BODY AND MICRO-ORGANISMS.

THAT certain races of animals and men, and certain individuals among these, are more refractory to disease than others is a fact which has long been known. Experience and observation have taught us, further, that the same individuals are at one time more resistant to disease than at another. This inborn or spontaneous refractory condition to an infectious disease is termed natural immunity, in contradistinction to that acquired by recovery from the disease.

In bacteria, we distinguish between the ability to produce poison and the power to multiply in the body. In animals and the higher plants we distinguish between immunity to poison and power to inhibit the development of bacteria.

In regard to variations in susceptibility, certain known facts have been accumulated. Thus, cold-blooded animals are generally insusceptible to infection from those bacteria which produce disease in warm-blooded animals, and *vice versa*. This is partly explained by the inability of the bacteria which grow at the temperature of warm-blooded animals to thrive at the temperature commonly existing in cold-blooded animals. But differences are observed not only between warm-blooded and cold-blooded animals, but also between the several races of warm-blooded animals. The anthrax bacillus is very infectious for the mouse and guinea-pig, while the rat is not susceptible to it unless its body resistance is reduced by disease and the amount of infection is great. The inability of the micro-organism to grow in the body of an animal does not usually indicate, however, an insusceptibility to its poison; thus, for instance, rabbits are less susceptible than dogs to the effects of the poison elaborated by the pneumococci, but these bacteria develop much better in the former than in the latter. Differences in susceptibility are sometimes very marked among different varieties of the same race of animals, as, for instance, between different kinds of rats and pigeons to anthrax. In animals, as a whole, it is noticed experimentally that the young of all species are less resistant to infection than the older and larger ones.

The difficulty experienced by the large majority of bacteria in developing in the tissues of the healthy body can be to a great extent removed by any cause which lowers the general or local vitality of the tissues. Among the causes which bring about such lessened resistance of the body are hunger and starvation, bad ventilation and heating, exhaustion from overexertion, exposure to cold, the deleterious effects of poisons,

bacterial or other, acute and chronic diseases, vicious habits, drunkenness, etc. Purely local injuries, such as wounds, contusions, etc., give a point of entrance for infection, and a point of less resistance, where the bacteria may develop and produce local inflammation. Local affections, such as endocarditis, may also afford an area of lessened resistance for the bacteria to seize upon. The presence of foreign bodies in the tissues in like manner predisposes them to bacterial invasion. Interference with free circulation of blood and retention in the body of poisonous substances which should be eliminated also tend to lessen the vitality. In these and other similar ways animals which are otherwise refractory may acquire a susceptibility to disease.

Increase of Resistance by Non-specific Means.—Just as all conditions which are deleterious to the body lessen its power of resistance to bacterial invasion, so all conditions which are favorable to it increase its resistance, and thus aid in preventing and overcoming infection. The internal use of antiseptics against bacteria has not proved successful, for the reason that an amount too small to inhibit bacterial growth is found to be poisonous to the tissue cells. The efficacy of quinine in malaria and mercury in syphilis is, possibly, an exception to the rule, but in both cases we are dealing probably with animal parasites, not with true bacteria. Such substances as nuclein and others contained in blood serum, when introduced into the body in considerable quantity, aid somewhat in inhibiting or preventing the growth of many bacteria. Even bouillon, salt solution, and small amounts of urine have a slight inhibitory action. The hastening of elimination of the bacterial poisons by free intestinal evacuation and encouragement of the functions of the skin and kidneys are also of some avail. The enzymes formed by certain bacteria have been found to exert a slight bactericidal action not only on the germs which have directly or indirectly produced them in the body, but also on other varieties. None of these enzymes are sufficiently protective to be of practical value nor equal in power to the protective substances formed by the tissues from the bacterial products.

Use of Local Treatment in Limiting Bacterial Invasion.—The total extirpation of the infected area by surgical means, if thoroughly carried out, removes the bacteria entirely; but, unfortunately, this procedure is rarely possible. When incomplete it is frequently helpful; but it may be harmful, for by creating tissue injury and exposing fresh wounded surfaces to infecion it may lead to the further development of the disease. Again, it is usually insufficient, for by removing only a portion of the bacteria it may leave those which have already reached the deeper tissues or blood to go on developing. In some cases, like anthrax and infection from bites of rabid animals, total or almost complete removal of the virus is possible, either by the knife or thorough cauterization, and will prevent a general infection or so lessen the number of bacteria in the body as to allow the bactericidal elements of its fluids to exterminate them. So also in tetanus, the invasion being limited, surgical interference may be of great use by removing not only

the bacilli themselves, but also that portion of their poison which has not as yet been absorbed from the tissues. The beneficial effects of opening an abscess, incising a cellulitis, or cleansing and drainage of the uterine cavity are well known. The retention of the poisonous products of the bacteria leads to their absorption, and then through their combining with the protective substances of the adjacent fluids the tone of the neighboring, and to a less extent of the general, tissues is lowered. This enables the bacteria to penetrate into tissues which would otherwise resist them. The mechanical effect of pressure on the walls of an abscess by its contents also aids the bacterial progress. Local bleeding and the application of cold probably act by lessening tension. The application of warmth increases the blood flow to the part, and so, when the general blood supply is bactericidal, as it often is, it acts favorably on the inflammation by destroying the bacteria. A peculiar effect of operative interference is noticed in the frequently observed beneficial result of laparotomy in tuberculous peritonitis.

Antiseptic solutions have the power of cleansing and rendering sterile the surfaces of a wound—that is, of preventing the introduction of infection. After infection has taken place, however, it is doubtful whether antiseptic washing has much more direct influence than simple cleansing, and it certainly can have no bactericidal effect at any distance from the surface, either direct or indirect. Certain infectious diseases which are comparatively superficial are probably benefited by antiseptic solutions, such as gonorrhœa, diphtheria, and other inflammations of the mucous membranes. Even here, however, it is impossible to do more than disinfect superficially, and in some cases any irritation of the tissues is apt to do more harm than good. In the superficial lesions of syphilis and tuberculosis the local use of antiseptics is sometimes of great value. In these diseases the irritant effects of the antiseptics which stimulate the tissues may also be beneficial.

Specific Immunity, or a Condition of the Body which Prevents the Development in it of One Variety of Micro-organisms or Renders it Unaffected by Their Bacterial Poisons.—The invasion of the body with more or less serious results by almost every micro-organism is followed by a condition which for a variable period and to a variable degree is deleterious to its further growth. It also may give rise to substances which neutralize the poisonous effects of the bacterial products. This specific immunity may take place in various ways:

1. Through recovery from disease naturally contracted or from infection artificially produced. According to the nature of the invading micro-organism this immunity may be slight, as after recovery from erysipelas or pneumonia, marked for a short period of time, as in diphtheria and typhoid fever, or prolonged, as after scarlet fever or syphilis.

2. By the injection of micro-organisms attenuated by heat, chemicals, or other means. In this case an infection of the animal is produced, of moderate severity, as a rule, and the immunity is not as marked and lasting as after recovery from a more serious attack; but it is, nevertheless, considerable. The inoculation of sheep with the attenuated

anthrax bacillus and the use of vaccination in man are examples of this method.

3. By the injection of the organisms into tissues where development will not take place, as the injection of typhoid bacilli or cholera spirilla into the subcutaneous tissues. Here the solution of the bacteria with the absorption of their products causes a mild chemical poisoning, with considerable resulting immunity.

4. By the injection of the chemical constituents of the dead bodies of bacteria and of the chemical products which they elaborate and discharge into the surrounding culture media during their life. Men as well as animals have been immunized by the injections of dead cultures of typhoid and anthrax bacilli and cholera spirilla, etc. A few days after infection with most parasitic bacteria the body resistance to the growth of the same organism is greatly increased; in other infections, however, it is but slightly augmented. This increased resistance is readily shown to be partly due to protective substances held in solution in the blood-serum and partly to the leukocytes.

5. By the injection of the blood serum of animals which have previously passed through a specific disease or have been inoculated with the bacterial products. The first, probably, to think of the possibility of effecting this was Raynaud, who in 1877 showed that the injection of large quantities of serum derived from a vaccinated calf into an animal prevented its successful vaccination. Héricourt, Richet, and others demonstrated the same thing for other diseases. The results obtained by Behring and Kitasato upon diphtheria and tetanus, where, indeed, the serum prevented the action of the poisons rather than the direct development of the bacteria, gave a still greater impetus to these investigations.

Suitable animals after repeated infections gradually accumulate in their blood considerable amounts of these protective substances, so that very small amounts of serum inserted in another animal will inhibit the growth of the bacteria or neutralize their products. Thus, 0.1 c.c. of a serum from a horse frequently infected by the pneumococcus will prevent the development in the body of a rabbit of one hundred times the fatal dose of very virulent pneumococci, and a few times a fatal dose of less virulent ones, the actual number as well as the virulence of the bacteria affecting the protective value of the serum.

These protective substances are found also in other fluids of the body than in the blood; they occur, indeed, in the substance of many cells to a greater or less extent.

The immunity produced by these five methods affects the entire body, as is natural, since the blood into which they are absorbed is distributed everywhere. When the immunity is but slight, infection may take place in the more sensitive regions, and still be impossible in those tissues having more natural resistance.

Passive as Contrasted with Active Immunity.—If the serum is injected into animals or man the immunity is greatest immediately after absorption, and then declines, being rather quickly (in several weeks or

months) almost entirely lost, so that repeated injections are required to maintain the immunity. This passive immunity is distinctly in contrast to the active immunity acquired after the introduction of bacterial products, where the tissues of the organism, in ways to us unknown, throw out, in response to the bacterial stimulus, inhibitory or antitoxic substances, or combine with the bacterial poisons to produce them. Here immunity is actually lessened for one or two days, and then is increased, and reaches its height a week or ten days after the injection, and then continues for a week or two, when it slowly declines again. The immunity produced by the transfer of serum from the immunized to the non-immunized is frequently called passive immunity and the immunity produced by infection active immunity.

Production of Antitoxin for Therapeutic Purposes.—If a greater quantity of protective substance than is required for the protection of the individual is desired in the blood, repeated injections of living or dead bacteria and their products are given, the doses being administered at short intervals, and in sufficient amount to produce a slight elevation of temperature and malaise. After several months of such treatment the blood is withdrawn, allowed to clot, and the serum then siphoned off aseptically and stored either with or without the addition of preservatives.

Testing of Protective Power of Antibacterial and Antitoxic Sera.—The serum is tested by mixing it with a certain number of times the fatal dose of a culture or its toxins whose virulence or toxicity is known, and then injecting this under the skin, in the vein, or into the peritoneum, according to the nature of the bacteria to be tested. The main point is that some definite method be carried out by which the relative value of the serum can be judged in comparison with other serums. As a rule, the value is stated in the number of fatal doses of culture or toxin which a fraction of a cubic centimetre of serum will prevent from destroying the animal. It is well to remember that with a living germ a multiple of a fatal dose is not as much more severe than a single dose as the figure would suggest. One thousand times a fatal dose of a very virulent micro-organism will be neutralized by several times the amount of serum which a single fatal dose requires, since in the case of very virulent living bacteria, whose virulence is due to their ability to increase, it is not the organisms which are introduced that kill, but the millions that develop from them.

Limitation of Curative Power of Serums.—As a rule, the serum has to be given before the bacteria introduced into the body have multiplied greatly. After that period has elapsed the serum usually fails to act. This is due to the fact that bactericidal power in serum is due to the combined effect of two substances, one only being contained in the injected serum. The serum loses all appreciable protective value as measured in test animals in the usual doses before the person is liable to infection. Repeated injections of serum continue this condition of immunity indefinitely.

Practical Value of Injections of Bactericidal Sera.—The use of serums having specific protective properties has been tried practically on a large scale in man as a preventive of infection. In susceptible animals injections of some of the very virulent bacteria, as pneumococci, streptococci, typhoid bacilli, and cholera spirilla, can be robbed of all danger if small doses of their respective serums are given before the bacteria have increased to any great extent in the body. If given later they are ineffective. For some bacteria, such as tubercle bacilli, no serum has been obtained of sufficient power to surely prevent infection. Through bactericidal serums, therefore, we can immunize against an infection, and even stop one just commencing; but as yet we cannot cure an infection which is already fully developed, though even here there is reason to believe that we may possibly prevent an invasion of the general system from a diseased organ, as by the pneumococcus from an infected lung in pneumonia. On the whole, the serums which simply inhibit the growth of bacteria have not given, as observed in practice, conclusive evidence of great value in already developed disease. This type of serum loses much of its bactericidal properties quickly and should not be used when kept for more than a few weeks.

Development of Antitoxins together with Bactericidal Substances.—Although the serum of animals which have been infected with any one of many varieties of bacteria is usually both antitoxic and bactericidal, still one form of these protective substances is usually present almost alone; thus antitoxic substances are present almost exclusively in animals injected with two species of bacteria which produce powerful specific poisons—viz., the bacilli of diphtheria and tetanus. When the toxins of either of these are injected in small amounts the animals after complete recovery are able to bear a larger dose without deleterious effects, and these doses in the more suitable animals can be gradually increased until a thousand times a previously fatal dose may be administered without any serious results whatever. To Behring and Kitasato we owe the discovery that this protecting substance accumulates to such an extent in the blood that very small amounts of serum are sufficient to protect other animals from the effects of the true extracellular toxins.

Except the diphtheria and tetanus bacilli a few only of the important parasitic bacteria attacking man produce these toxins and thus become capable of causing the production in the body of antitoxins, and even these do it to a far less extent than those of tetanus and diphtheria. Following them is the plague bacillus, and then the cholera spirilla, the typhoid bacilli, the streptococci, etc. These latter bacteria when injected excite more of the substances which inhibit bacterial growth than of those which neutralize their toxins.

Antitoxin a Preventive.—Antitoxin prevents the poisonous action of toxin. It does not restore the cells after they have been injured by the toxin; it is, therefore, like the bactericidal substances, a preventive rather than a cure. We find, experimentally, that a very much smaller amount of antitoxin will neutralize a fatal dose of toxin in an animal, if given before or at the same time, than if given only shortly after it.

An animal already profoundly poisoned by the toxin is unaffected by any amount of antitoxin.

Duration of Immunity.—The antitoxins of diphtheria and tetanus are gradually eliminated from the body after their injection or after their production from toxin injections. After the usual immunizing dose the duration of immunity is only from two to six weeks, the period differing in each individual. The elimination of the antitoxin takes place partly through the urine and other secretions, and it is partly destroyed in the body. An animal which has been highly immunized will retain considerable amounts of antitoxin for from two to four months.

Stability of Antitoxins.—The different antitoxins vary as to their stability thus: that of diphtheria is somewhat more stable than that of tetanus. Kept aseptically in cold and dark storage, and protected from access of air, the more resistant antitoxins may be preserved sometimes for a year or two with very little deterioration in strength. At other times, however, from unknown causes, they are gradually destroyed, so that there may be a loss of about 10 per cent. per month. A serum requires, therefore, to be tested every few months if we wish to be assured of its strength in antitoxin. Preservatives, such as carbolic acid, trikresol, etc., alter antitoxins only very slightly when in dilute solution, but in strong solution they partially destroy them. Heat up to 62° C. does not injure them greatly, but higher temperatures alter them.

Method of Administration.—Antitoxins and bactericidal substances are absorbed to a very slight extent only when taken by the mouth—certainly less than 1 per cent. They must, therefore, be introduced subcutaneously or intravenously to enter the body. The antitoxic serum does not act against the bacteria directly, but, by neutralizing their poisons, it prevents them from acting as irritants to the cells, and so the soil for the growth of the bacteria becomes unsuitable, and they cease to develop.

CHAPTER XV.

NATURE OF THE PROTECTIVE DEFENCES OF THE BODY AND THEIR MANNER OF ACTION—EHRLICH'S "SIDE CHAIN" THEORY.

THE fluids and tissues of the animal body under the normal conditions of life are, as we have seen, not only unsuitable for the growth of the great majority of the varieties of bacteria, but even bactericidal to the living organisms and antitoxic to their poisons.

In seeking to account for the bactericidal property of the blood, which to a greater or less extent affects all bacteria, we cannot find it either in the insufficient or excessive concentration of the nutritive substances, nor in the temperature, nor in the reaction; for although some of these conditions may be unsuitable for some bacteria, they are all suitable for many, and thus cannot constitute the fundamental explanation of either natural or acquired immunity. We are thus driven to the conclusion that the body fluids and cells contain substances which are deleterious to most or all of the bacteria. As to the origin of these substances, we may conceive that they may be either regularly produced by certain types of the many varieties of body cells, or that they may only be produced when bacteria or other foreign cells or their substance invades the body. When formed we can conceive that they may remain unaltered in the fluids for a long period of time or be quickly eliminated or destroyed.

Bactericidal Properties of the Blood.—The bactericidal effect upon most bacteria of the blood serum, noted by Nuttall in 1888, is now undisputed, and is readily shown by the fact that moderate numbers of bacteria when inoculated into freshly drawn blood usually soon die, and this destruction may be so rapid that in a few hours none of millions remain alive. Even when some of the bacteria survive there is for a time a decrease in the number living. That this effect of the blood is due to substances in the serum, and not due to serum as such, can be proven by the fact that not only by injecting bacteria into the blood and peritoneal cavity, but also when the bacteria are placed in the animal body after being enclosed in capsules or into serum contained in test-tubes, the bacteria are killed even if they have previously grown outside the body in inactive blood serum. Bacteria have also been injected into a vein carefully ligated above and below, and here, without coagulation, the blood exerts bactericidal properties. The germicidal effect of any sample of blood serum on different varieties of bacteria is unequal and can be watched outside of the body. Here mixed with it some species of bacteria die quickly, and some lose only a portion of their number, those remaining alive after a time rapidly increasing. The

number of bacteria introduced is of great importance, for the serum with its contained substances is capable of destroying only a certain number, and after that loses its bactericidal properties.

Thus the following test was carried out:

No. of bacteria in 1 c.c. fluid.	Amount of serum added.	Approximate number alive after being kept at 37° C.		
		One hour.	Two hours.	Twenty-seven hours.
30,000	0.1 c.c.	400	2	0
100,000	0.1 c.c.	5,000	1,000	200,000
1,000,000	0.1 c.c.	400,000	2,000,000	10,000,000

After the proof given by Pasteur and his pupils as to the existence of acquired immunity, attempts to explain it were made. Pasteur formulated his exhaustion theory, in which an analogy was drawn between an infection in a living animal and the exhaustion of food in a culture media by the growth of a bacterium. The knowledge that injections of toxins or bacterial protoplasm was followed by the production of antibodies disproved his theory in the sense he understood it, but Ehrlich's theory of the necessity of suitable cell receptors to allow of union of poison to cell suggests the possibility that natural immunity is sometimes due to the lack of suitable receptors or sensitive substances.

Chauveau, like Pasteur, starting from facts observed in a culture, considered that acquired immunity was due to substances retained in the body after recovery from an infection which were noxious to bacteria. Here, again, later information has changed the explanation; so that we know that it is not substances left by bacteria, but deleterious substances produced by the body cells through the stimulus of the bacterial products.

During these earlier years Metchnikoff perceived that the infected host was too little considered, and he drew attention to the role of the leukocytes. His original theory of immunity is based on the observations that leukocytes frequently take up bacteria injected into the blood. Metchnikoff held that the virus was destroyed in the interior of certain mesodermic cells by a process of digestion.

At the same time that this theory was being developed another was gradually being evolved. It was noticed that the bacteria injected into the blood and tissues disappeared. Nuttall showed that bacteria are destroyed in cell-free serum but that this property of the serum is destroyed by heating it at 56° C. Buchner made many experiments, and finally elaborated his alexin theory of immunity. He showed that bacteria absorbed these bactericidal substances. Later, Bordet, Ehrlich, and others established that the alexin of Buchner was really a mixture of two substances of which one, named "immune body," is developed as the result of the injection of foreign cell substance and is resistant to heat, and the other, named "complement," is present in the blood of normal animals, is not increased by injection, and is unstable. Neither one of these substances alone destroy bacteria, while together they do. Ehrlich pointed out the similarity of complements to the true toxins, such as those produced by the diphtheria and tetanus bacilli.

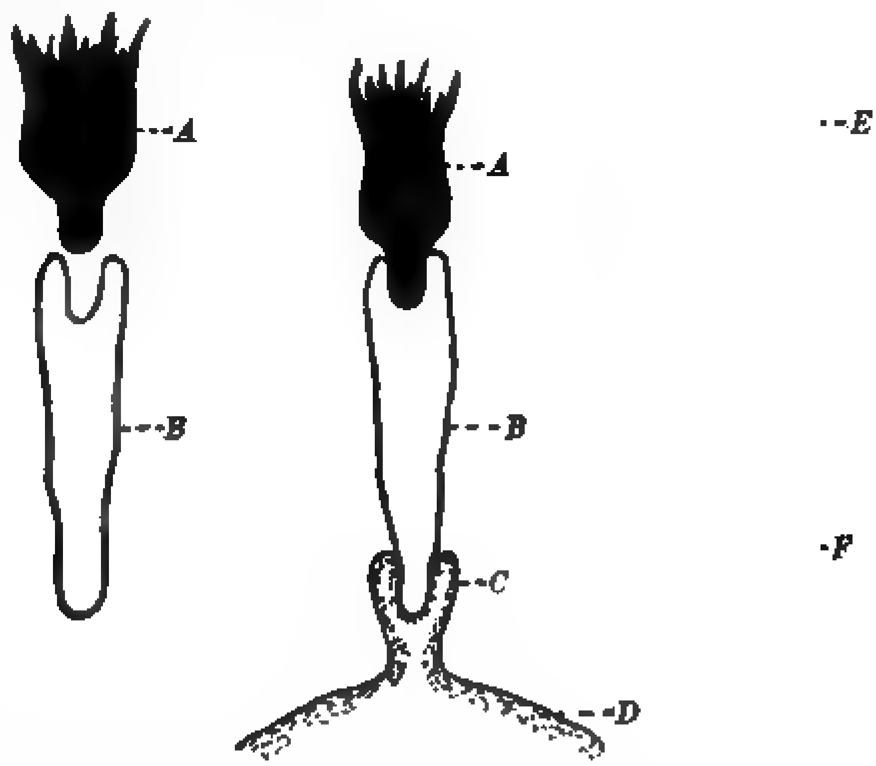
During the investigations on the bactericidal substances of the blood the discovery of the antitoxins was made by Behring and Kitasato, and the nature of toxins was investigated by Roux, Ehrlich, and others.

Ehrlich's Theories.—Ehrlich's researches led to the development of his theories on immunity which have had a powerful influence upon all later investigations in this field. His pupil and colleague, Wassermann, explains them in the following words:

Ehrlich began by observing that of the many poisonous substances known to us only a comparatively small number existed against which we could truly immunize—*i. e.*, obtain specific antibodies in the blood serum of the immunized organism. Let us look at two poisons which are very similar in their physiological action, for example, strychnine and tetanus poison, both of which excite spasms through the central nervous system. It is really curious that the injection of one, strychnine, produces no antibody whatever in the serum, while the injection of the other, the tetanus poison, causes the formation of the specific tetanus antitoxin. Ehrlich says that this is because these substances enter into entirely different relations with the cells of the living organism. The one substance, strychnine, merely enters into a loose combination with the cells of the central nervous system, so that it can again be abstracted from these cells by all kinds of solvents—*e. g.*, by shaking with ether or chloroform. The combination, therefore, is a kind of solid solution, such as has been shown for the staining with aniline dyes. The tetanus poison, on the contrary, Ehrlich says, is firmly bound to the cell; it enters the cell itself, becoming a chemical part of the same, so that it cannot again be abstracted from the cell by solvent agents. He compares this process to the assimilation of nutrient substances. Hence the difference between these two substances could be likened to that between saccharin and sugar. Both substances taste sweet, but, despite this similarity in one of their physiological actions, they behave very differently toward the cells of the organism. Saccharin simply passes through the organism without entering into a firm combination—*i. e.*, without being assimilated—and is therefore no food. Its sweetening action is due to a mere contact effect on the cells sensitive to taste. Sugar, on the contrary, is actually bound by the cells, assimilated and burnt, so that it is a true food. Ehrlich says that the first requirement for every substance against which we can obtain a specific serum must be its power to enter into such a combination with certain particular cells in the living organism. The substance must possess a definite chemical affinity for certain parts of the organism. Hence, in each substance against which we can specifically immunize, Ehrlich assumes a group of atoms which effects the specific binding to certain cells, the *haptophore group* (Fig. 67, *F*). Corresponding to this is a group in the cell of the living organism *C*, the *receptor group*, with which the haptophore group combines. The latter is entirely distinct from that part of the substance which exerts the physiological or pathological effect, in toxins, for example, from the group which is the carrier of the poisonous action, the so-called *toxophore group E*, or in fer-

ments, from the group which exerts the ferment action, the *zymophore group.* Both groups, haptophore and functional, are independent of each other, and their separate presence can easily be demonstrated because the functional group—*e. g.,* in poisonous toxins the toxophore group—is more readily destroyed by heat than the haptophore group. Thus by heating a toxin for some time to 60° to 65° C. substances will be obtained which are no longer poisonous, but which still possess the binding, haptophore, group. In the case of toxins such substances are called *toxoids.* Ehrlich further stated that the finer mechanism of the formation of specific substances was this: that the haptophore group was bound to the receptor of the living organism owing to a specific affinity. As a result of this the receptor is lost to the living organism, disposed of, and a biological law formulated by Weigert

FIG. 67



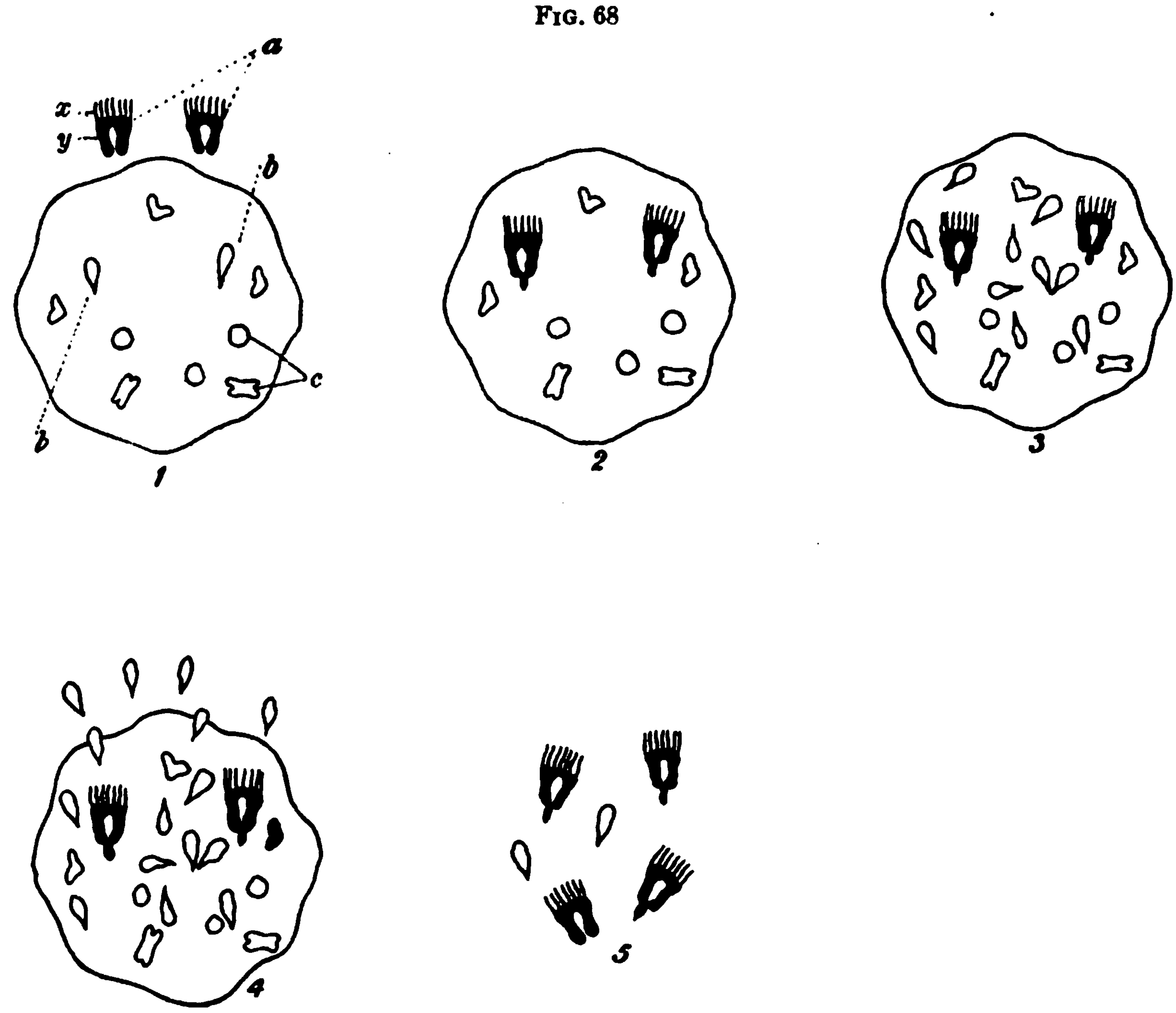
Graphic representation of receptors of the first and third orders and of complement as conceived by Ehrlich: *A*, complement; *B*, intermediary or immune body; *C*, cell receptor; *D*, part of cell; *E*, toxophorous group of toxin; *F*, haptophorous group.

now comes into action, the law of supercompensation; that is, the organism seeks to replace this defect, but in doing so not merely replaces the receptors in question, but, according to Weigert, produces more of them than were previously present (Fig. 68). The conditions are somewhat like those seen in the callus after a fracture, in which the organism likewise does not produce just the amount of bone previously present; there is always an overproduction.

In this way, Ehrlich says, such a large number of one type of receptors are produced by the organism that these become too much for the same; they are then thrust off into the blood, and these free receptors circulating in the blood constitute the specific antibodies. Ehrlich therefore believes that the specific antibodies in the serum are nothing else than receptors for which the substance employed in immunization

possesses specific affinity. Hence, the same substance which, so long as it remains in the organ, attracts the toxin and makes it possible for that to exert its poisonous action on the organ, this same substance acts as a protection when it circulates outside of the organ; for then it satisfies the affinity of the poison's haptophore group while still in the blood, preventing the poison molecule from acting on the organ itself.

FIG. 68



Graphic representation of Ehrlich's theory of the production of antitoxin and the neutralization of toxin: *a*, diphtheria toxin molecule; *x*, toxophore atom group; *y*, haptophore or combining group; *b*, cell receptors with affinity for diphtheria toxin; *c*, other cell receptors.

1. Cell with its receptors. Outside of cell, free toxin molecules.
2. Toxin molecules combined with the cell receptors having affinity for diphtheria toxin.
3. After three days, showing multiplication of cell receptors similar to those combined with toxin.
4. After four days, excess of receptors cast off in the blood.
5. Toxin molecule neutralized by combining with free receptors in blood of immunized animal or in an animal into which blood with free receptors had been transferred.

In the formation of the specific antibodies we must therefore distinguish three stages (Fig. 68):

The binding of the haptophore group to the receptor (2).

The increased production of the receptors following this binding (3).

The thrusting off of these increased receptors into the blood (4).

A considerable part of Ehrlich's theories upon toxins and antitoxins have been confirmed experimentally; for example, the presence of the separate toxophore and haptophore groups and the existence of atom-groups with specific binding properties in all substances with which we can immunize.

Up to the present time the mechanism of the increased production of receptors following the binding of the haptophore group to the receptor has not been experimentally proven. Wassermann, however, believes that he and Bruck have now been successful in this. Although I consider his interpretation as most interesting, yet both this and the reasoning from it are far from proven.

They employed a tetanus poison kept since 1896. This poison was originally very toxic. In the course of years, however, owing to the damaging influence of this long standing, that is, owing to the action of light, the oxygen of the air, etc., it had become so weak that it was no longer toxic at all. They were able to give a guinea-pig 1 c.c. without producing tetanus. Nevertheless, haptophore groups had remained intact, as could readily be proved, for this non-poisonous tetanus toxin was still able to bind tetanus antitoxin—*i. e.*, thrust-off receptors. When they injected rabbits with this non-poisonous tetanus toxoid in increasing doses, and then examined the blood serum of the animal, they found *not a trace of tetanus antitoxin*. They considered the absence of antitoxin in this experiment could have either of two causes:

1. It might be that the toxoid no longer produced any physiological effect whatever in the organism; or

2. Although it still caused an increase in the receptors in the cells, these increased receptors remained in them, and were not thrust off into the blood.

In order to decide this question they first determined as nearly as possible the exact quantity of fresh tetanus toxin which constituted a fatal dose for guinea-pigs of a definite size. If, now, the action of the tetanus toxoid was such that in the living organism it was *not* bound to the receptors, it should be possible to prove this experimentally.

Their line of reasoning was as follows: Were they to inject first the toxoid, and shortly after, say, in one to two hours, the fresh toxin, they would in such an animal have to increase the fatal dose—*i. e.*, they would require more tetanus toxin to kill this animal than an untreated one, because owing to the previous toxoid injection the most susceptible cell receptors had already been occupied. Provided Ehrlich's theory was correct, so that this binding of the toxoid really occurred, the conditions should be entirely different when, instead of injecting the toxin shortly after the toxoid, they waited somewhat longer, one to three days, and then injected the fresh tetanus toxin. For in that case Weigert's law should come into play and the receptors have commenced to increase in numbers—*i. e.*, the organ would now possess more sensitive groups than before. This would have to manifest itself in such fashion that in contrast to the first experiment the fatal dose of fresh tetanus toxin could now be decreased; in other words, a small dose would now tetanize the animal in a shorter time.

As a matter of fact, the experiments yielded results which were exactly like those deduced theoretically as just described. They injected a guinea-pig with some of the non-poisonous toxoid, and then, one hour later, with the tetanus toxin. They found that much more

toxin was required to kill this animal than a normal guinea-pig of equal size. If, on the contrary, they waited one to three days, it was found that then a dose of tetanus toxin which would not even tetanize an untreated guinea-pig was sufficient to kill this one.

If their explanation of the above experiments is correct, then we have for the first time proof of the existence of the first two of the three stages demanded by Ehrlich's theory. The first stage, the binding of the haptophore group, is demonstrated with the toxoid; the second stage, the increased production of receptors, is demonstrated by the second series of experiments just described; the third stage is the thrusting off of the receptors. Close attention to these experiments will show that with a completely non-poisonous toxoid no receptors were thrust off at all. The serum of the rabbit treated with this toxoid contained no antitoxin whatever. Hence it follows that the thrusting off of the receptors into the blood serum does not necessarily follow from their overproduction, but that something additional is required. This "something," which we may term a stimulus, is a function of the toxophore group. Because of these experiments Wassermann conceives the mechanism of the presence of the specific antibodies in the serum to be as follows: 1. The haptophore group is bound by the receptor. 2. The consequence of this binding is a proliferation of the receptors. In this stage, however, they are still attached to the organ. 3. In order that they be thrust off, a certain stimulus is required. The haptophore group is incapable of exerting this, so that, in the case of tetanus toxin, this is exerted by the toxophore group. These experiments, although important, do not appear to me to establish as much as Wassermann conceives. There is no proof that additional receptors in the cells of an animal so sensitive as a guinea-pig would in fact make it respond to a smaller dose of toxin. It would be equally easy to argue that it would be less sensitive, since fewer cells might absorb all the toxin. The fact that the cells combining with the toxoid yielded up no antitoxin might be taken to prove that the cells specifically attacked were not the ones that produced the antitoxin.

Other Explanations of the Production of Antitoxins.—Gruber along with others consider that the antitoxins are not normal constituents of the cells but simply secretions of the cells under the stimulus of the toxin, and the cells secreting are not the cells sensitive to the toxin, but others—*e. g.*, the blood-producing organs. Gruber claims that the power to poison highly immunized animals with toxin is against Ehrlich's ideas. This objection can be met in several ways. Ehrlich believes that the receptors while still in the cell may have a greater affinity for the toxin than after being thrown into the circulation. Gruber claims that the latent period in toxin poisoning is due to the slow absorption of the toxin by the circulation, and does not need the elaborate explanation of the toxophore group acting through the haptophore. The idea that antitoxin is simply the toxin modified has been given up for one reason because the antitoxin developed is several thousand times enough to neutralize the toxin injected.

One objection against the Weigert-Ehrlich hypothesis of overproduction of antitoxin by the specifically attacked cells is that while the animals are still showing tetanic symptoms the receptors of the still diseased cells are supposed to have been reproduced, as shown by antitoxin production. This is answered by Weigert that while the more important cell atom groups are still suffering, the groups producing the receptors may have recovered. This supposition is difficult to prove or disprove.

The idea of Weigert, that the cells are biologically altered so as to continue to make receptors (antitoxin) after the cessation of the injections, is not in accord with the observations made by us, as there is uniformly a great drop in antitoxin ten days or two weeks after the cessation of the fresh stimulus of renewed injections. We have also shown that by partially neutralizing toxin before injecting it into animals, it is possible to excite the cells to produce as much antitoxin from the first as from any later injections.

We know from the researches of Meyer and Ransom that tetanus *poison* is not absorbed by the affected nerve cells by way of the blood and lymph channels, but reaches them by way of the nerves. Following its injection the tetanus poison ascends in the axis cylinder of the nerves to the motor cells. The tetanus *antitoxin*, unlike the toxin, is not a neurotrophic substance, but always follows the blood and lymph channels. In adrenalin we possess a substance which is able strongly to contract the capillaries and thus to block the blood absorption path of a particular area. Proceeding from these facts, Wassermann and Bruck devised the following experiment: Tetanus toxin and antitoxin were mixed in such proportions that the mixture was entirely innocuous to animals. If this mixture was injected into the hind paw of a guinea-pig no tetanus developed. When, however, some adrenalin was injected into the hind paw of a similar-sized guinea-pig, and after waiting a few minutes until the capillaries had contracted, there was injected a recent mixture of tetanus toxin and antitoxin, typical tetanus was produced. What happened was this: that the channel of absorption for the tetanus antitoxin had been blocked, while that for the toxin, the nerve path, was open. The toxin had therefore torn loose from its combination and followed its course to the central nervous system, where it produced tetanus.

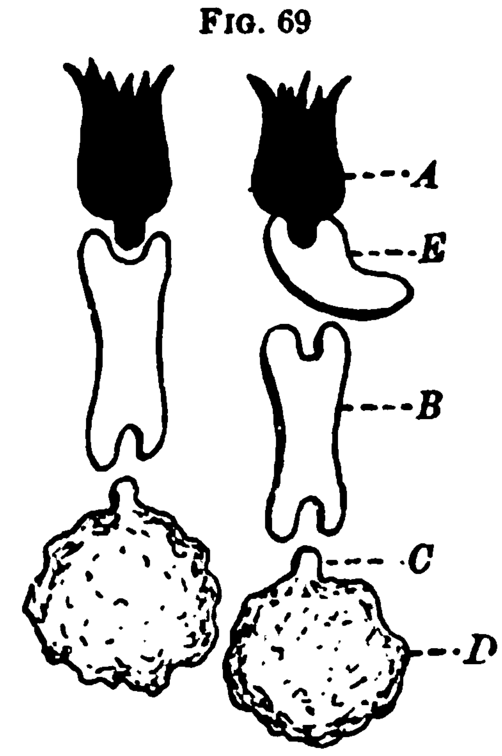
This experiment, however, succeeds only within a certain period—*i. e.*, not over an hour after mixing the toxin and antitoxin. If we wait a longer time, say, three or four hours, it will be found that even in the adrenalin animal no tetanus is produced, because by this time the combination, previously a loose one, is so firm that the substances can no longer be torn apart. This union can be hastened by employing more tetanus antitoxin, for with an excess of antitoxin even after only half an hour it is impossible by means of adrenalin to loosen the tetanus toxin. This experiment, therefore, shows that the tetanus toxin-antitoxin combination is at first a loose one, and that the affinity becomes firmer and firmer with time. It also shows the possibility of slightly

increasing this affinity by means of an excess of antitoxin, a point of considerable practical value in serum therapy.

Antibacterial Action. THE ACTIVE SUBSTANCES IN BACTERICIDAL AND BACTERIOLYTIC SERUM.—The first important fact noted which suggested that a bactericidal serum produced its action through more than one substance was the discovery that while the power of a fresh bactericidal serum to kill bacteria in the test-tube is lost by heating it to 60° C., yet injected into the peritoneal cavity or blood of a living animal the power is still exerted. The same is true if in the test-tubes to the heated serum there is added some fresh normal serum which is itself incapable of destroying the bacteria. From these observations the fact became gradually apparent that the bactericidal property of a cell-free serum depended upon two components.

Different investigators have applied to them different names. The one which is resistant to heat, which attaches itself directly to bacteria,

FIG. 69



Graphic representation of amboceptor or receptors of the third order and of complement, showing on left the immune body uniting complement to foreign cell and on right the action of anticomplement, binding complement: *A*, complement; *B*, intermediary body; *C*, receptor; *D*, cell; *E*, anticomplement.

even at low temperatures, and is increased during immunization, is called sensitizing substance, interbody, amboceptor, or immune body. The other, which is sensitive to heat, which is present in the healthy normal serum, is not increased during immunization, and which unites with the bacterial protoplasm only at temperatures considerably above the freezing point, is called alexin, or complement.

The immune body attaches itself to the bacterial substance, but does not appreciably harm the cells. The complement destroys the cell after the immune body has made the cell vulnerable.

According to Ehrlich the immune body first unites with the protoplasm of the cell and this develops in the immune body an affinity for the complement and the two unite. (See Fig. 69.) He believes that it is through the immune body that the complement exerts its action on the cell.

Others believe that the immune body and complement do not directly unite. It appears as if the immune body injured the cell membrane

and so allowed the complement to penetrate the cell substance or that, as the French put it, changes its nature so it can combine with its complement. Muir has shown that when cells are saturated with both immune body and complement that the addition of fresh cells causes a splitting off of immune body but not of complement. This throws further doubt upon the direct union of immune body and complement. Most of the experiments which have been made with the purpose of clearing up these difficult problems have been made upon red blood cells. Here the absorption of the immune bodies at low temperatures and the lack of noticeable injury until the complement is added, at a temperature of 20° to 30° C., is very striking. The immune bodies are very numerous and fairly specific in their action. The complement substance is much less specific and, although probably multiple, each variety acts upon widely different bacteria after they have united with the immune body. There is little reason to think that the complement of one animal is any more capable to attack bacteria prepared by immune bodies developed in its blood than by immune bodies developed in some other species.

The building of immune bodies in the infected animals is believed by most to take place the more rapidly the more virulent the infecting organisms are. In our experiments this has not been evident. Increase of virulence for one species of animal does not mean increase for all animals; so that the animal upon which the virulence is tested must be the same variety as the one being immunized to draw conclusions.

Origin of Bactericidal Substances.—Ehrlich and his followers consider the immune bodies to be built up in the same way as the antitoxins. They are cell atom-groups, which are similar to those which were attacked by the bacterial substance and which were overproduced as the cell attempted to replace what had been destroyed or bound up.

Their source must apparently be attributed to the cells, but probably only certain cells produce them. The red blood cells, for instance, seem rather to destroy than to increase them. The nuclein derived from the cells, although it has a general bactericidal action, and may enter into the complements (alexins), has different properties, and so cannot itself be one of these bodies. The cells which have abundant nuclear substance, such as the leukocytes and lymph cells, seem especially to be a source, and Metchnikoff asserts their pre-eminent role as the producers of both complements and immune bodies. Buchner and others have found that through the irritation of bacterial filtrates the leukocytes were attracted in great numbers to the region of injection, and that the fluid here, which was rich in leukocytes, was more bactericidal than that of the blood serum elsewhere. Some claim to have demonstrated that along with increased leukocytosis there is a general increase in the complement in the blood; still, it has not yet been positively established that the complement is derived solely from the leukocytes, nor from all leukocytes, and a mere increase in them does not always mean an increase in the complement. Immune bodies appear to be more abun-

dant in the spleen and the hæmopoietic organs and also to appear there first during the process of immunization.

The Part Played by the Leukocyte in Immunity.—The original theory of Metchnikoff, that the leukocytes were the only actual protective bodies which warded off disease, and that they did this by attacking the bacteria was founded on the now well-known fact that certain of the white cells possessed the power of taking up into themselves pathogenic bacteria, and that they were there destroyed. It was later observed that these cells had the property of taking from the blood many lifeless foreign elements, thereby keeping the blood channels free of foreign particles.

The question thereby arose as to whether these cells engulfed and then killed the bacteria, or whether perhaps other substances killed or prepared them before the cells took them up. It is now known that the bacteria first unite with substances in the serum and thus are prepared for phagocytosis. This union does not kill the bacteria. The leukocytes and the chemical substances of the blood thus both play an important part. The death of the bacteria also liberates positive chemotactic substances, and the disintegration of the white blood cells gives rise to bactericidal bodies. We find that phagocytosis is most marked when the disease is on the decline or the infection mild, but is usually absent in rapidly increasing infection. This would seem to indicate that the course of the infection is often already determined before the leukocytes become massed at the point of its entrance. The first determining influence is given by the condition of the tissues and the amount of bactericidal substances contained in them, and then, later, in cases where the bacteria have been checked, comes the additional help of the leukocytes. If the tissues are wholly free of bactericidal and sensitizing substances, neither they nor the leukocytes, nor both combined, can prevent the bacterial increase. The simple absorption by the cells of bacteria is not necessarily a destructive process. Metchnikoff believes that the polymorphonuclear leukocytes are especially antibacterial in relation to acute infections. The large phagocytes are conceived to deal chiefly with the resorption of tissue cells and with immunity to certain chronic diseases, such as tuberculosis.

Opsonines[1] or Substances which Prepare the Bacteria for the Leukocytes.—Wright, Neufeld, and others have studied the substances in the blood which prepare the bacteria for the leukocytes. The interesting fact has been noted that the white blood corpuscles of the non-immune and the actively immune have the same phagocytic characteristics toward bacteria, the differences in their apparent activity being due to the substances in the serum which, similar to the immune body, combine with the receptors in the cells.

If the washed cells from the immune patient are added to salt solution they have no more effect upon the bacteria used to immunize than do those from a susceptible person, while those from the latter put into the serum of the former act energetically. Heating destroys all opsonines

[1] Greek "Opsono"—I cater for.

according to Wright, but some, only, according to Neufeld. For many infections, such as those due to the staphylococci, streptococci, and pneumococci, immunity seems to depend largely on the opsonines and leukocytes.

The opsonic power is measured by placing bacteria in a mixture of the patient's serum and a saline emulsion of fresh white blood cells. These are usually obtained from the fluid collecting after injecting an aleuronat emulsion suspended in a thin starch paste into the pleural cavity of a rabbit or other animal. The fluid is withdrawn twelve to eighteen hours after the injection. In order to separate the leukocytes from the pleural fluid they are washed in normal salt solution and centrifugalized. We have found that suitable leukocytes can be obtained very readily from the horse. The blood is received directly into a flask containing some 10 per cent. solution of sodium citrate, and in such amount as to make a 1:10 solution. This mixture does not clot. The red cells sink to the bottom and the supernatant fluid is centrifugalized.

After placing at 37° C. for from fifteen to sixty minutes the average number of bacteria taken up by the white corpuscles is determined by examining stained preparations. Wright noticed that there was no opsonic power in the fluid in an abscess cavity, and that even while the blood as a whole might have it, that of some organ or portion of the body might lack it.

Deflection of the Complement.—It frequently happens that when the addition of a small amount of immune serum renders a normal serum more bactericidal a greater addition robs it of all bactericidal power. This is explained by Neisser and Wechsberg to be due to a locking up of complement by excess of immune body. The subject is in need of further study.

Multipartial or Polyvalent Sera.—According to Ehrlich's theory, every immunizing group in a substance corresponds to a countergroup of the fitting receptors in the organism. Bacteria are not homogeneous masses, but are made up of various molecules which differ biologically from one another. Conforming to this, the antisubstances, immune bodies (antitoxins, agglutinins, etc.), which appear in a serum are made up of the sum of the antibodies which correspond to these partial elements in the bacterial body. These separate groups are called "partial groups." An immune serum, therefore, consists of the partial groups which correspond to the separate partial elements of the bacterial body. We are further able to show that these partial elements in one and the same bacterial species are not the same for all the bacteria of that species. Thus one culture of streptococci or of *bacillus coli* may have a few partial elements which differ from those of another culture. What is the consequence of this? The consequence will be that when we immunize with a culture *a* of such bacteria we shall obtain a serum which acts completely on this culture, for in this serum all the partial elements present in culture *a* are represented. If, however, we employ culture *b*, *c*, or *d*, which perhaps possess other partial elements, we

shall find that the serum does not completely affect these cultures. As already stated, such a condition of things is met with in inflammations due to streptococci and other bacteria, and is, therefore, of considerable practical importance. It is because of this fact that a serum acts best only in a certain percentage of cases. In order to overcome this difficulty in persons infected with these bacterial species we have no choice but to make sera, not by means of *one* culture, but by means of a number of different strains of the same species. The result of this will be that, corresponding to the various partial elements in these different cultures, we shall obtain a serum containing a large number of the partial groups. Such a serum will then exert a specific action on a large number of different cultures, but not quite as great an influence on anyone as if only that variety had been injected.

In other words, the development and the closer analysis of the problem of immunity, especially during the past few years, have shown us that we must make use, more than heretofore, of so-called *polyvalent* or multipartial sera. In the serum therapy of streptococcus infections, of dysentery, etc., the production of such multipartial sera is an advantage in practice. Owing to these partial groups also, a serum—*e. g.*, antityphoid serum—can specifically affect a closely allied species of bacterium, like *bacillus coli*, for example. For it is known that closely related species of bacteria, such as typhoid and colon bacilli, possess certain partial groups in common, and a serum is thus produced which to a certain extent acts on both species. This constitutes what is known as the "group reaction."

CHAPTER XVI.

THE NATURE OF THE SUBSTANCES CONCERNED IN AGGLUTINATION.

THE agglutinating substances, which develop in animals because of bacterial infection, have proven of such value in the identifying of bacteria and the detection of bacterial infection that a knowledge of them is of great practical as well as theoretical importance. (See pages 81–83 for technique of investigation.)

The agglutinins were discovered by Gruber and Durham. Their effect on bacteria can be observed either macroscopically or microscopically. For example, if a serum from an animal which has passed through a typhoid infection is added to a twenty-four-hour culture of typhoid bacilli, and the mixture placed in a thermostat, the following phenomenon will be noticed: The bacteria, which previously clouded the bouillon uniformly, clump together into little masses, settle to the sides of the test-tube, and gradually fall to the bottom until the fluid is almost entirely clear. In a control test, on the contrary, to which no active serum is added the fluid remains uniformly cloudy. The reaction is completed in from one to twelve hours. If the reaction is observed in a hanging drop, it is seen that the addition of the active serum first produces an increased motility of the bacteria which lasts a short time and is followed by a gradual formation of clumps. Frequently one sees bacteria which have recently joined a group make violent motions as though they were attempting to tear themselves away; then they gradually lose their motility completely. Even the larger groups of bacteria may exhibit movement as a whole. After not more than one or two hours the reaction is completed; in place of the bacteria moving quickly across the field, one sees one or several groups of absolutely immobile bacilli. Now and then in a number of preparations one sees a few separate bacteria still moving about among the groups. If the reaction is feeble, either because the immune serum has been highly diluted or because it contains very little agglutinin, the groups are small and one finds comparatively many isolated and perhaps also moving bacteria. It is essential each time to make a control test of the same bacterial culture without the addition of serum. Under some circumstances the reaction proceeds with extraordinary rapidity, so that the bacilli are clumped almost immediately. By the time the microscopic slide has been prepared and brought into view, nothing is to be seen of any moving or isolated bacteria, and only by means of the control test is it possible to tell whether the culture possessed normal motility. As to the nature of these phenomena a number of theories have been

advanced. As in the case of the immune body there is positive proof that the agglutinin combines directly with substances in the bacterial body.

In some cases the agglutinins are active even in very high dilutions. Thus in typhoid patients and typhoid convalescents a distinct agglutination has been observed in dilutions of 1:5000, and this action persisted for months, though not, of course, in the same degree. Even normal blood serum, when undiluted, often produces agglutination. But the specific agglutinins, which are formed only in consequence of an infection, are characterized by this, that they produce agglutination even when the serum is highly diluted, and, furthermore, that after this dilution the action is specific—*i. e.*, the dilution of cholera immune serum agglutinates only cholera bacilli, typhoid immune serum only typhoid bacilli, etc. This specificity, however, as will be shown later, is not always absolute.

The agglutinating substances when mixed with bacteria are bound to the agglutinable substances in them, the two bodies effecting a loose combination very like toxin and antitoxin. By chemical means it is possible to again separate the agglutinin from the bacteria and use it to agglutinate bacteria anew.

It was formerly assumed that agglutination was a *prerequisite for bacteriolysis*. This, however, is not so, for both in cholera and in typhoid immunity bacteriolytic substances have been observed without agglutinins, and agglutinating substances without bacteriolysins.

Of the three antibodies mentioned, serum therapy has thus far made use of the antitoxins; whereas, in serum diagnosis the bacteriolysins and above all the agglutinins are used. *Serum diagnosis by means of these two substances was possible only because they had proven themselves in general as specific.*

Precipitins.—If we inject an animal with albuminous bodies of the greatest variety, substances will usually appear in the blood which possess distinct relations to these bodies. They manifest themselves by their power to precipitate the albuminous bodies from dilute solutions in a test-tube. These antibodies are therefore called *precipitins*. A phenomenon discovered by R. Kraus is probably to be separated from this precipitin action on albumins. This author showed that the serum of a rabbit immunized against typhoid produces a precipitate in the bacterial-free filtrate of a bouillon typhoid culture. This fact has been verified by a number of investigators and found to extend also to other species of bacteria. The precipitins are divided like agglutinins into having group and specific action.

Characteristics of Agglutinins.—As considered by Ehrlich's school, the agglutinin consists of a stable-combining group and an unstable-precipitating group. The agglutinable molecule is also believed to consist of two groups, one stable that combines with the agglutinins and one labile that gives the completed reaction. Agglutinins changed by heat, acids, and other influences become agglutinoids, which are comparable to toxoids, complementoids, etc.

The union of agglutinin with receptors in bacteria is a chemical reaction, and is quantitative. Before agglutination occurs sodium chloride must be present as it enters into the combination. The amount of bacteria in the emulsion used to test the amount of agglutinin must, therefore, be known. An emulsion one hundred times as dense as another would require one hundred times as much agglutinin to give an equally complete reaction. Agglutinin acts upon dead bacteria.

Many things affect the agglutinable substance in bacteria. Grown in bouillon for three days at 37° C., bacilli requires three times as much agglutinin to give an equal reaction as if grown at 37° C. for one day. This difference is partly due to bacterial substance passing out into the culture medium, which combines with the agglutinin.

Heat diminishes the agglutinability of bacteria when above 60° C. Dreyer found that if a twenty-four-hour bouillon culture of bacillus coli required 1 part of agglutinin to agglutinate it, then if heated to 60° C. it required 2.3 parts; if to 80° C., 18 parts; if to 100° C., 24.6 parts. He found the surprising fact that long heating of the culture restored its ability to be agglutinated by smaller amounts of agglutinins.

Heated thirteen hours to 100° C., the culture was agglutinated by 4 parts. Dreyer's explanation of this result is that agglutinin-fixing substance is dissolved out by the prolonged heating.

Heating the serum above 60° C. injures the agglutinin slightly, above 70 C. greatly, and above 75° C. destroys it. Weak and strong acids agglutinate bacteria while medium acidity does not. Alkalies inhibit agglutination.

The nature of agglutinoids and the means by which they inhibit agglutination is at present little understood. It is important to remember that in concentrated serum agglutination may fail because of their action, while in higher dilutions of the serum agglutination may take place readily.

The growth of bacteria in fresh blood or its equivalent inhibits the development of agglutinable substance in bacteria. Bacteria should not be grown on such media when they are to be used in agglutination tests. Even ascitic fluid broth has some effect.

Group Agglutination.—Many varieties of bacteria have among the different substances composing their bodies some of which are common to other bacteria which are more or less allied to them. These substances all exciting agglutinins we have such a serum acting on a number of varieties. These agglutinins are called, therefore, group agglutinins. If a typhoid or paratyphoid serum possess a high degree of activity—*i. e.*, ability to agglutinate even in large dilution—it may happen that with lesser dilution it may also agglutinate the two related bacilli. Thus, in two cases the infecting paratyphoid bacilli type B were agglutinated 1:5700; typhoid bacilli, however, only 1:120, while paratyphoid bacilli type A were not agglutinated at all. In two other cases I observed an agglutination of paratyphoid type B with a dilution 1:40, while typhoid bacilli were agglutinated with 1:300 and over. Korte has frequently observed that typhoid sera agglutinate not only typhoid bacilli, but also one or both varieties of paratyphoid, even

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when a simultaneous infection with paratyphoid was excluded. This agglutination for the other bacilli was in some cases quite marked, though there was no uniformity whatever. Since he found that, conversely, in paratyphoid infection the serum possesses a fairly strong agglutinating action on typhoid bacilli, Korte advises that in every case of typhoid all three bacteria be tested for agglutination, so that, according to the strongest agglutinating action, one can decide which infection is present. If in practice it is immaterial whether this point be decided, the agglutination with paratyphoid need only be undertaken when the typhoid agglutination is absent.

In all this we are dealing with the same phenomenon which undoubtedly plays a role in the agglutination with blood of icteric patients, the so-called *group agglutination*, as it was first termed by Meinhard Pfaundler.[1] In other words, while agglutinins may be nearly, if not quite, specific in their action a serum which produces agglutination may be far from being so. As a rule, the agglutination with the infecting agent is by far the strongest—*i. e.*, it proceeds even in high dilutions—whereas other bacteria require a stronger concentration. The bacteria which are agglutinated by one and the same serum need not at all be related in their morphological or other biological characteristics, as at first assumed. Conversely, micro-organisms which, because of the characteristics mentioned, are regarded as entirely identical or almost so, are sharply differentiated by means of their agglutination. In other words, the "groups" arrived at by means of a common agglutination have no relation to species as the term is usually employed. Thus, according to Stern, certain varieties of proteus and of staphylococci excite the production of sera which exert marked agglutinating powers also on typhoid bacilli, although otherwise we do not regard these three micro-organisms as at all related. Because of this lack of absolute specificity the *serum diagnosis of infection or identification of bacteria* has value only when very carefully tested.

The Relative Development of Specific and Group Agglutinins.—The study of a large number of series of agglutination tests obtained from young goats and rabbits injected chiefly with typhoid, dysentery, paradysentery, paracolon, colon, and hog-cholera cultures has shown that there is considerable uniformity in the development of the specific and group agglutinins. The specific agglutinins develop in larger amount in the beginning, being in the second week usually from five to one hundred times as abundant as the group agglutinins. Later the total amount of the group agglutinins tends to approach more nearly to that of the specific, and reach as high as 50 per cent. In a number of tests carried out by us we found that many group agglutinins supplement specific ones in their action, causing by their addition an increased agglutinating strength. In our experience the variety of micro-organism used for inoculation is, if equally sensitive, agglutinated by the combined specific and group agglutinins produced through its stimulus

[1] Ueber Gruppenagglutination und das Verhalten des Bacterium coli bei Typhus, Münch. med. Wochenschrift, 1899, No. 15.

in a higher dilution than any micro-organism affected merely by the group agglutinins. It is true that bacteria not injected were at times agglutinated in higher dilutions than the variety injected; this, if not due to greater sensitiveness, was on account of normal group agglutinins present in the animal before immunization. In horses and adult goats it was found that before injections were commenced there was often a great accumulation of agglutinins for bacteria and especially for members of the dysentery, paradysentery, and colon groups, so that the estimation of the development of specific agglutinins was a matter of great difficulty except through careful absorption experiments. For this reason untreated horse serum is a very dangerous substance to use in differentiating the intestinal bacteria. This is clearly brought out in the record given of the tests made of two horses. The great height to which the group agglutinins may rise is seen in the following table:

TABLE I.

Agglutinin in the Serum of a Horse Injected with Paradysentery Bacillus. Type, Manila Culture.

	After 18 injections.			After 21 injections.		
Culture.	1:3000	1:5000	1:10,000	1:3000	1:5000	1:10,000
Paradysentery type, Manila . .	++	—	—	++	++	++
Colon B. X.	++	++	—	++	++	++

The great amount of agglutinins acting upon the colon bacillus X. is remarkable. A serum is here seen to be acting in dilutions as high as 1:10,000 upon a culture possessing very different characteristics from the one used in the injections.

Although a considerable proportion of the group agglutinins acting on colon bacillus X was undoubtedly due to the stimulus of the injections of the Flexner paradysentery culture, still a portion of them was probably due to other causes. In Table II. is seen the marked accumulation of agglutinins which may occur in a horse before injections are begun and the results of injection nutrient bouillon which had been prepared from meat in the usual way.

TABLE II.

	A young horse before inoculation.				The same one week after being injected with one litre of bouillon.			
Culture.	1:100	1:500	1:1000	1:5000	1:100	1:500	1:1000	1:5000
Dysentery B., Japan . .	+	—	—	—	++	—	—	—
Paradysentery, Mt. Desert	+	—	—	—	++	—	—	—
" Manila .	++	++	++	—	++	++	++	—
Colon B. X. . . .	++	+	—	—	++	++	+	—

The fact of most importance which appears in this table is the abundant agglutinins which may be found in the serum of a horse which has never received bacterial injections.

Three rabbits injected with nutrient bouillon developed agglutinins for the paradysentery and some of the colon bacilli. In one rabbit's serum the Manila culture agglutinated in dilutions of 1:150.

TABLE III.

Serum from Two Horses at the End of One Year, during which Weekly Injections were Given.

Culture.	Dysentery B. (Shiga). 1:500	1:1000	1:5000	Paradysentery (Manila). 1:500	1:1000	1:5000
Dysentery B., Japan	+	–	–	++	–	–
Paradysentery B., Mt. Desert	+	–	–	++	–	–
" " Manila	++	++	–	++	–	–

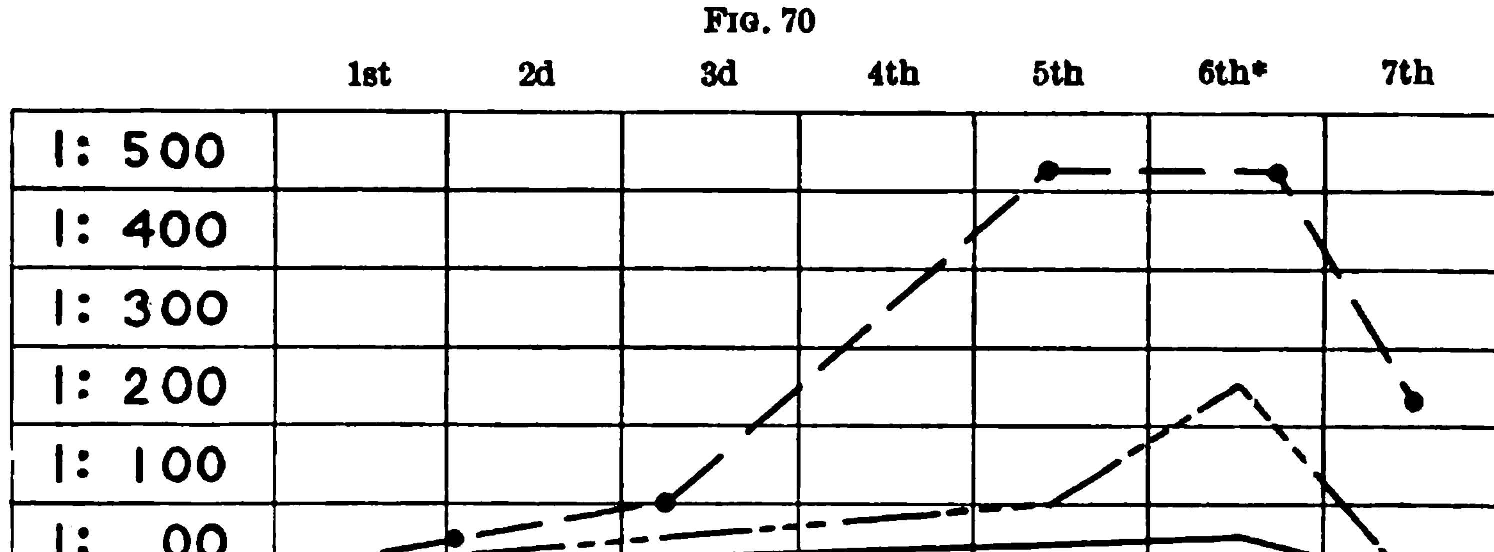
It is interesting to note that the serum from the horse receiving the Shiga culture agglutinates the paradysentery culture in higher dilutions than the Shiga culture. In the other serum all cultures were agglutinated in equally high dilutions in spite of the fact that the paradysentery type had been injected.

THE RELATIVE ACCUMULATION OF THE GROUP AND SPECIFIC AGGLUTININS.—This is seen to vary for the different types and at different times. For the Manila culture of Flexner, which is nearest to the colon in its characteristics, the specific agglutinins were in the serum of an animal which had received injections of the Manila cultures at the end of the third month six times as abundant as the group agglutinin acting on the Maine culture of Park, which represents a type farther removed from the colon. At the end of the fourth month they were fourteen times as abundant. For the dysentery bacillus (Shiga) the development of agglutinins was the least.

Another point of interest is that the proportional amount of agglutinins from the different cultures varied at different times. If on tests made of a single bleeding we had attempted to draw conclusions as to the relative development of specific and group agglutinins between the cultures, we would have had an imperfect view. Many conflicting statements in literature are undoubtedly due to this lack of appreciation of the variability in the relative amount of these two types of agglutinins during a long process of immunization.

The development of group agglutinins for the three dysentery types caused by the injections of a colon bacillus:

FIG. 70

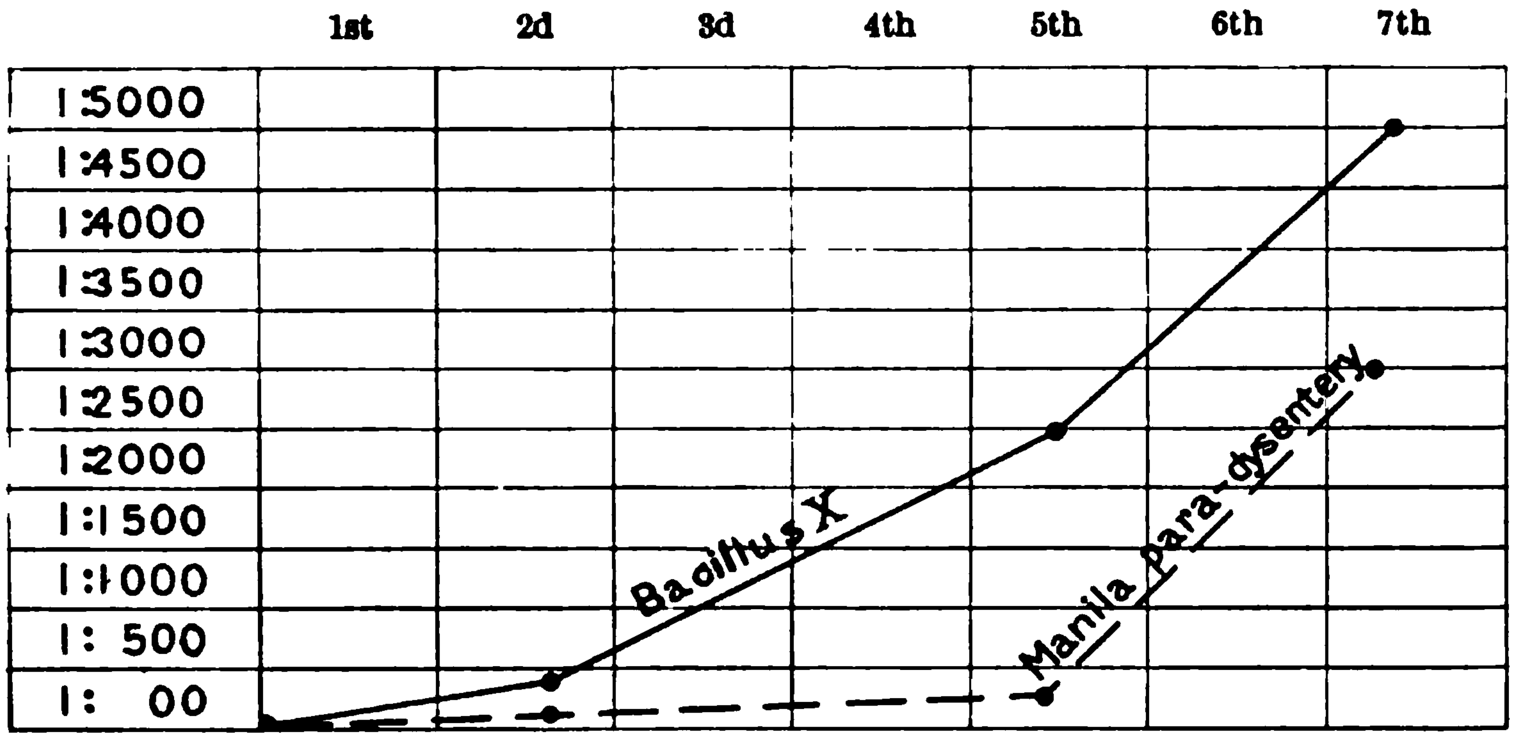


The rise and fall of common and specific agglutinins during seven months in a rabbit injected with the Manila culture after it had been heated to 115° C.

——— ——— Colon bacillus X.
— — — — — Paradysentery type (Manila).
— - - - — - - - — Paradysentery type (Mt. Desert).
- - - - - - - - - - - - Dysentery type (Japan).
● Test dates for all four sera.

* Injections stopped.

FIG. 71



Similar conditions to those noted in previous chart, except that a young goat has been used for the injections of the colon bacillus X. The great accumulation of common agglutinins for the para-dysentery bacillus in the third month of the injections of the bacillus X is very striking.
● Tests made.

The Use of Absorption Methods for Differentiation between Specific and Group Agglutinins due to Mixed Infection and to a Single Infection.— It is now well established that if an infection is due to one micro-organism there will be specific agglutinins for that organism and group-agglutinins for that and other more or less allied organisms. If infection is due to two or more varieties of bacteria, there will be specific agglutinins for each of the micro-organisms and group agglutinins produced because of each of them.

The above facts have been demonstrated by several investigators. The following experiments selected from those reported by Castellani[1] well illustrate these points: A rabbit immunized to B. typhi agglutinated B. typhi 1:5000, B. coli (31) 1:600. After saturation with B. typhi all agglutinins were removed for both micro-organisms. A rabbit immunized to both B. typhi and B. coli (31) agglutinated B. typhi 1:4000, B. coli (31) 1:1000. (After saturation with B. typhi the serum did not agglutinate B. typhi, but B. coli (31) 1:900.) After saturation with B. coli it failed to agglutinate B. coli (31), but still agglutinated B. typhi 1:4000.

From these and other experiments Castellani drew the important conclusions:

1. The serum of an animal immunized against a certain micro-organism, when saturated with that micro-organism, loses not only its agglutinating power for that organism, but also for all other varieties that it formerly acted upon. Saturated with the others, its action upon the first is reduced little or not at all.

2. The serum of an animal immunized against two micro-organisms, A and B, loses its agglutination when saturated with A only for A. Saturated with A and B it loses its agglutinating power for both.

3. These facts may be applied to the diagnosis of an unknown mixed

[1] Zeitschrift f. Hyg., Bd. xl., S. 17.

infection. Suppose, for instance, the serum from a typhoid case agglutinates both the laboratory cultures of the typhoid bacilli and those of a variety of the colon group. We saturate the serum with typhoid bacilli. If the serum loses its agglutinating power for the typhoid bacillus only, it is a case of mixed infection with both the typhoid bacillus and the type of colon bacillus used in the test. If the serum loses its agglutination for both the B. typhi and the B. coli, then it is a pure typhoid infection, the B. coli having been agglutinated by the group-agglutinins produced because of the typhoid infection.

The conclusions Castellani derived from the facts stated in paragraphs 1 and 2 are not warranted, because of the fact that bacteria absorb group agglutinins produced by other varieties of bacteria and which agglutinins may not appreciably affect them. The agglutinins in the serum of the supposed case of typhoid fever which agglutinated the test culture of B. coli and were absorbed by B. typhi were not, it is true, produced by the variety of B. coli of the test culture, but they may have been produced, and in fact probably were, by some other variety of B. coli. The B. typhi is less apt to produce abundant group agglutinins for B. coli than are other varieties of B. coli, and it absorbs the group agglutinins produced by many varieties of the B. coli for other bacteria.

The results of a number of experiments carried out by us demonstrate this. The following tables give the outcome of several experiments:

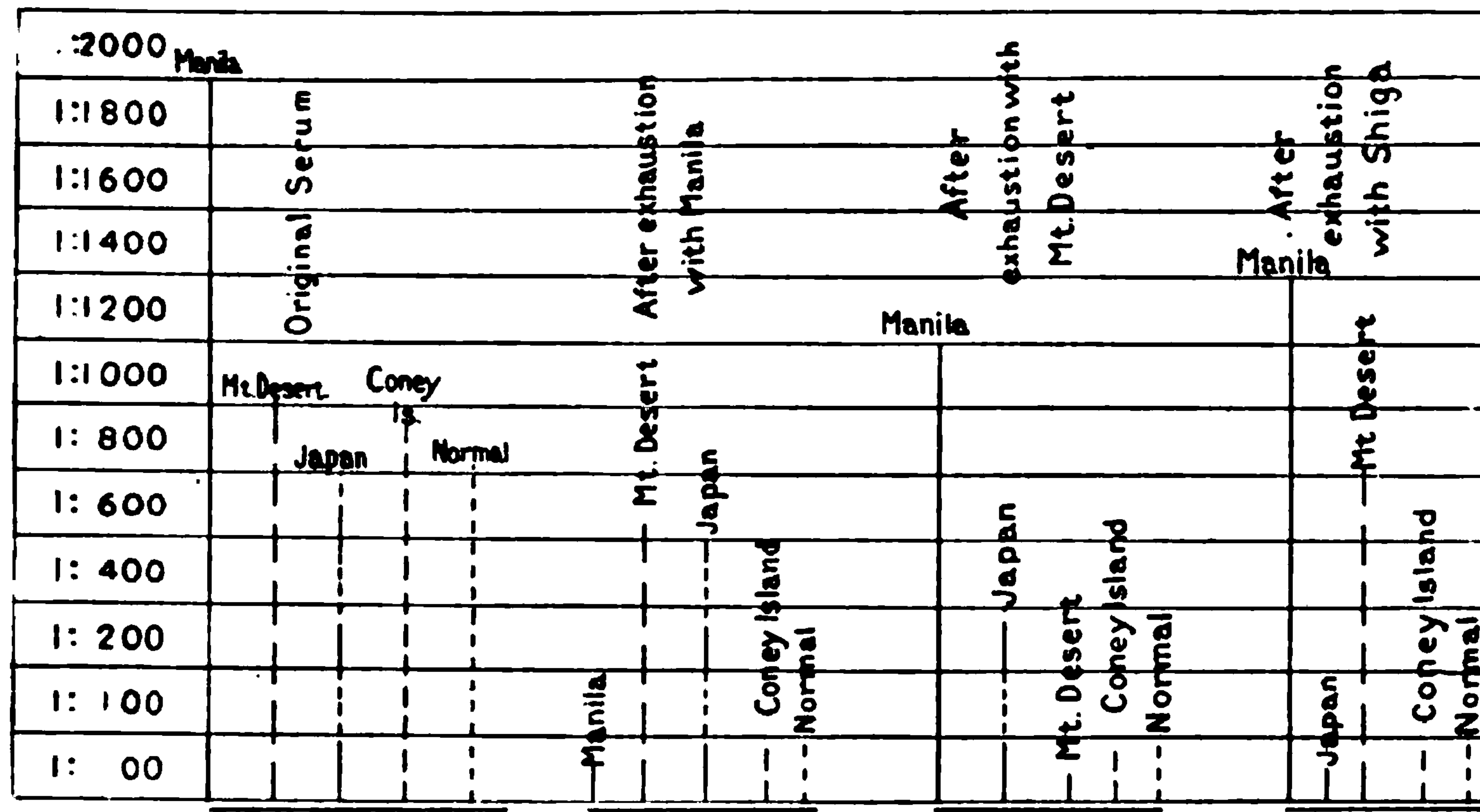
Absorption by the Typhoid Bacillus of Group Agglutinins Acting upon a Number of Varieties of B. coli which were Produced by Another Variety of B. coli.

Agglutination by Serum of Rabbit Immunized to Colon Bacillus X.

	Before addition of typhoid bacilli.	After attempt at absorption with typhoid bacilli at 22° C.
Colon bacillus X	600	600
" " 1	500	20
" " 2	500	30
" " 3	250	30
" " 4	250	10
" " 5	10	less than 10
" " 6–18	less than 10	" " 10
Typhoid "	" " 10	" " 10

The absorption tests were carried out by adding the bacilli from recent agar cultures to a 10 per cent. solution of the serum in a twenty-four-hour bouillon culture. The mixture was allowed to stand for twenty-four hours at about 22° C. It was found that a simple dilution of serum when left at 37° C. rapidly deteriorated. Thus, in an extreme instance a serum positive at 1:1500, when diluted with bouillon or salt solution 1:25 and left at 37° C. for twenty-four hours, lost 30 to 40 per cent. of its strength; at 22° C. it lost 15 to 20 per cent. Left for three hours only, the loss was only 5 to 10 per cent.

FIG. 72



Showing the effect of saturating with bacilli of types Shiga-Manila and Mt. Desert, a serum from a horse which had received combined injections of dysentery bacilli of the three types. Note that the Manila type removed almost all the specific and group agglutinins acting upon its own type and the group agglutinins acting upon the Coney Island and normal types, leaving the specific agglutinins for types Shiga and Mt. Desert. The same is true for types Shiga and Mt. Desert when they were used.

———— Manila paradysentery. — — — — Mt. Desert paradysentery.
— - - - — - - - Japan paradysentery. — — — and - - - - Atypical paradysentery.

The absorption method simply proves, therefore, that when one variety of bacteria removes all agglutinins for a second the agglutinins under question were not produced by that second variety.

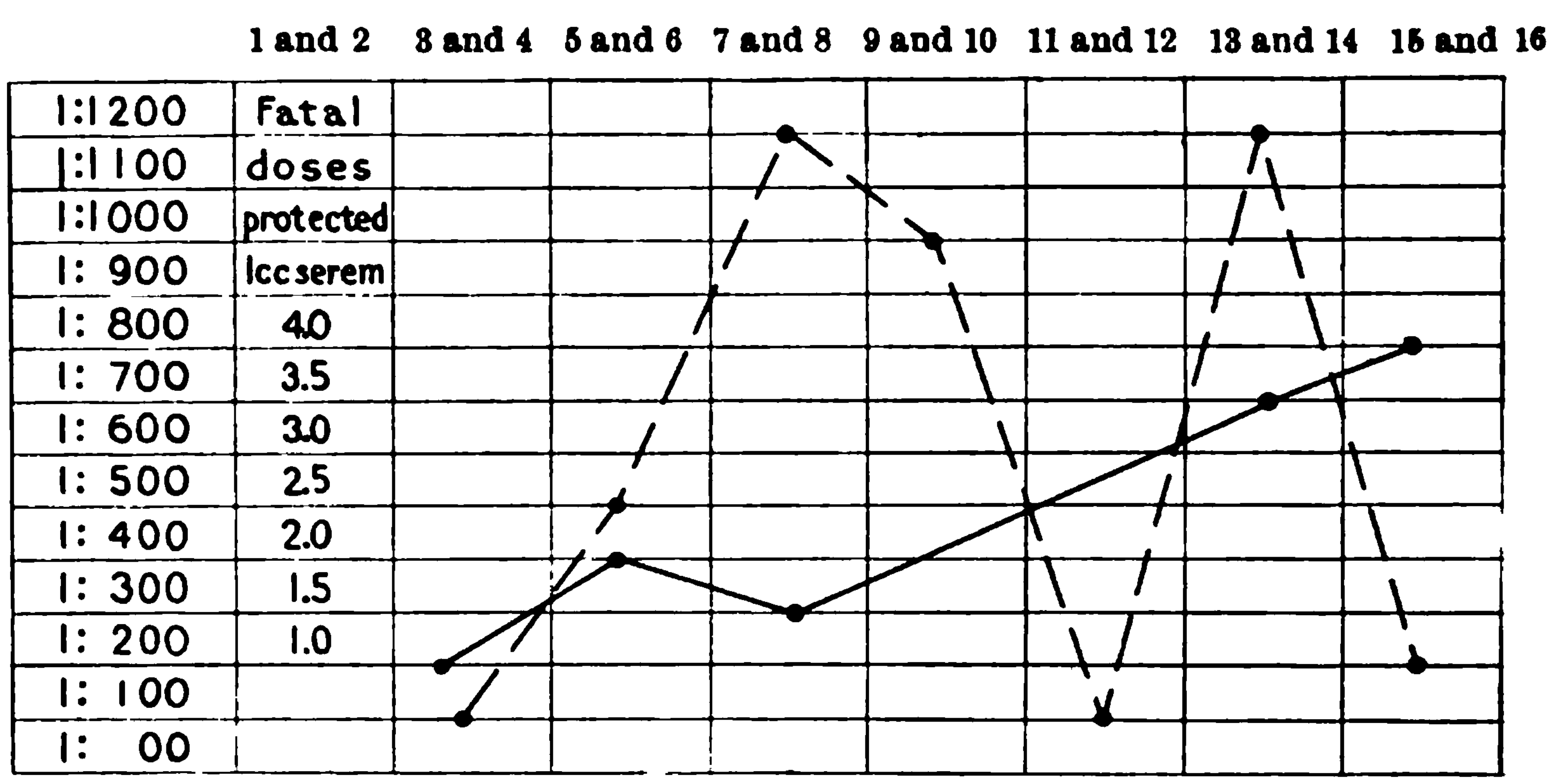
Loss of Capacity in Bacteria to be Agglutinated or to Absorb Agglutinins Because of Growth in Immune Sera.—The loss of these characteristics by growth in sera has been demonstrated by Marshall and Knox. The experiments of Dr. Collins and myself are recorded because they were undertaken in a slightly different way and also because a certain number of confirmatory observations are of value.

The maltose fermenting paradysentery bacillus of Flexner was grown on each of eleven consecutive days in fresh bouillon solutions of the serum from a horse immunized through oft-repeated injections of the bacillus. The solutions used were 1.5, 4, and 15 per cent. The serum agglutinated the culture before its treatment in dilutions up to 1:800, and was strongly bactericidal in animals. After the eleven transfers the culture grown in the 15 per cent. solution ceased to be agglutinated by the serum and ceased to absorb its specific agglutinins. The cultures grown in the 1.5 and 4 per cent. solutions agglutinated well in dilutions up to 1:60 and 1:100 and continued to absorb agglutinins. The recovery of the capacity to be agglutinated was very slow when the culture was from time to time transplanted on nutrient agar. After growth for sixteen weeks, during which it was transplanted forty-three times, it agglutinated in dilutions of 1:200. The culture grown in 4 per cent. agglutinated 1:500, and the one in 1.5 per cent. 1:800. This diminution and final cessation of development of agglutinable substance in bacteria grown in a serum rich in agglutinin and immune

bodies is interesting both as showing the variation of the bacteria and as one means of adapting themselves to resist destruction, since the bacteria which ceased to produce agglutinable substance probably also produced less substance with affinity for other antibodies. This inhibition of the production of agglutinable substance was also very noteworthy in the case of pneumococci grown in serum media.

Relation between Agglutinating Bactericidal Power.—In spite of proof to the contrary many good observers hold to the belief that there is some relation between the agglutinating and the bactericidal strength of a serum. The tests we carried out on the serum of a number of horses showed no such relation. In Fig. 73 are recorded a number of comparative tests during a period of sixteen months. For the tests of the bactericidal power of the serum we are indebted to Dr. Mary E. Goodwin. She also showed that there was a production of group as well as specific immune bodies in the animals receiving prolonged injections. The results of her experiments will be published later.

FIG. 73



Relation of agglutinative power to bactericidal. Horse injected with Manila culture over a period of sixteen months.

— — — — Agglutination index. ——— Bactericidal index.

● Tests dates.

Variation in the Agglutinating Strength of a Serum.—There is usually a continued increase in the amount of agglutinin in the blood of an infected person from the fourth day until convalescence and then a decrease. At times, however, there is a marked variation from day to day, so that it may be abundantly present one day and almost absent the next.

PART II.

BACTERIA PATHOGENIC TO MAN INDIVIDUALLY CONSIDERED.

CHAPTER XVII.

THE BACILLUS AND THE BACTERIOLOGY OF DIPHTHERIA.

Historical Notes.—The specific contagious disease which we now call diphtheria can be traced back under various names to almost the Homeric period of Grecian history. The Greeks believed that it had been communicated to their country from Egypt. The description of the pharyngeal and laryngeal manifestations of this disease left by Aretæus leaves no doubt that it was of diphtheria that he wrote. From time to time during the following centuries we hear of epidemics both in Italy and in other portions of the civilized world which indicate that the disease never absolutely ceased. The disease early crossed to America, and in the New England States we get clear accounts of its ravages.

In 1765 Home, a Scotchman, tried to show that "croup" and pharyngeal diphtheria were different diseases, and this subject remained under controversy until it was settled, through bacteriological examinations, that while most cases were undoubtedly diphtheria, a few were not.

In 1771 Bard, an American, supported the opposite theory from Home, considering the process the same wherever located. His observations upon diphtheria were very important and accurate.

In 1821 Bretonneau published his first essay on diphtheria in Paris and gave to the disease its present name. His observations were so extensive and so correct that little advance in knowledge took place until the causal relations of the diphtheria bacilli and their associated micro-organisms to the disease began to be recognized. Since then the combined clinical, bacteriological, and pathological studies have sufficed to make diphtheria one of the best understood of diseases.

The Diphtheria Bacillus.

Discovery.—In the year 1883, bacilli which were very peculiar and striking in appearance were shown by Klebs to be of constant occurrence in the pseudomembranes from the throats of those dying of true

epidemic diphtheria. One year later, Loeffler published the results of a very thorough and extensive series of investigations on this subject. He found the bacillus described by Klebs in many cases of throat inflammations which had been diagnosticated as diphtheria. He separated these bacilli from the other bacteria present and obtained them in pure culture. When he inoculated the bacilli upon the abraded mucous membrane of susceptible animals more or less characteristic pseudomembranes were produced, and frequently death or paralysis followed with characteristic lesions.

In 1887–88 further studies by Loeffler, Roux, and Yersin added to the proof of the dependence of diphtheria on this bacillus. It was found that, while no other forms of bacteria were constantly met with, the diphtheria bacilli were present in all characteristic cases of diphtheria, and that these bacilli possessed the morphological, cultural, and pathogenic qualities of those described by Klebs and Loeffler. The results of these investigations have since been confirmed by a great number of combined clinical and bacteriological observations both in animals and human beings.

Human Inoculation Experiments.—A very instructive accidental experiment was carried out under our observation some years ago. One of the investigators unintentionally drew quite a quantity of a bouillon culture of a virulent diphtheria bacillus into the throat, and two days later characteristic diphtheria of a serious type developed. Similar accidents have happened in two other laboratories. In view of known facts, we are now justified in saying that the name diphtheria should be applied, and exclusively applied, to that acute infectious disease usually associated with pseudomembranous affection of the mucous membranes which is primarily caused by the bacillus diphtheriæ of Loeffler.

Morphology.—When cover-glass preparations made from the cultures grown on blood serum are examined, the diphtheria bacilli are found to possess the following morphological characteristics: The diameter of the bacilli varies from 0.3 to 0.8μ and the length from 1 to 6μ. They occur singly and in pairs (see Figs. 74 to 81) and very infrequently in chains of three or four. The rods are straight or slightly curved, and usually are not uniformly cylindrical throughout their entire length, but are swollen at the end, or pointed at the ends and swollen in the middle portion. The average length of the bacilli in pure cultures from different sources frequently varies greatly, and even from the same culture individual bacilli differ much in their size and shape. This is especially true when the bacilli are grown in association with other bacteria. The two bacilli of a pair may lie with their long diameter in the same axis, or at an obtuse or an acute angle. The bacilli possess no spores, but have in them highly refractile bodies, some of which are the starting point for new bacilli.

Staining.—The Klebs-Loeffler bacilli *stain* readily with ordinary aniline dyes, and retain fairly well their color after staining by Gram's method. With Loeffler's alkaline solution of methylene blue, and to a less extent with other weak staining solutions, the bacilli from blood-

serum cultures, especially, and from other media less constantly, stain in an irregular and extremely characteristic way. (See Fig. 74.) The bacilli do not stain uniformly. In many cultures round or oval bodies, situated at the ends or in the central portions, stain much more intensely than the rest of the bacillus. Sometimes these highly stained bodies are thicker than the rest of the bacillus; again, they are thinner and surrounded by a more slightly stained portion. The bacilli stain in this peculiar manner at a certain period of their growth, so that only

FIG. 74

FIG. 75

FIG. 74.—One of very characteristic forms of diphtheria bacilli from blood-serum cultures, showing clubbed ends and irregular stain. × 1100 diameters. Stain, methylene blue.

FIG. 75.—Extremely long form of diphtheria bacillus. This culture has grown on artificial media for four years and produces strong toxin. × 1100 diameters.

FIG. 76

FIG. 77

FIG. 76.—Diphtheria bacilli characteristic in shapes, but showing even staining. In appearance similar to the xerosis bacillus. × 1100 diameters. Stain, methylene blue.

FIG. 77.—Non-virulent diphtheria bacilli, showing stain with Neisser's solutions, supposed to be characteristic of virulent bacilli. Bodies of bacilli in smear, faint brown; points, dark blue.

a portion of the organisms taken from a culture at any one time will show the characteristic staining. In old cultures it is often difficult to stain the bacilli, and the staining, when it does occur, is frequently not at all characteristic. The same round or oval bodies which take the methylene blue more intensely than the remainder of the bacillus are brought out still more distinctly by the Neisser stain.

The Neisser stain is carried out by placing the cover-slip smear of diphtheria or other bacilli in solution No. 1 for from two to three sec-

onds, and then, after washing, in No. 2 for from three to five seconds. The bacilli will then appear either entirely brown or will show at one or both ends a dark-blue, round body. With characteristic diphtheria bacilli, taken from a twelve to eighteen hours' growth on serum, nearly all will show the blue bodies (Fig. 76), while with the pseudotype (Fig. 83, page 197), to be described hereafter, few will be seen.

The solutions are as follows:

No. 1.

Alcohol (96 per cent.)	20 parts.
Methylene blue (Grübler)	1 part.
Distilled water	950 parts.
Acetic acid (glacial)	50 "

No. 2.

Bismarck brown	1 part.
Boiling distilled water	500 parts.

The Neisser stain has been advocated in order to separate the virulent from the non-virulent bacilli, without the delay of inoculating animals; but in our hands, with a very large experience, neither the Neisser stain nor other stains, such as the modifications of the Roux stain, have given much more information as to the virulence of the bacilli than the usual methylene-blue solution of Loeffler. A small percentage of virulent bacilli fail to take the Neisser stain, and quite a few non-virulent pseudodiphtheria bacilli show the dark bodies. In New York there are also a large number of bacilli which seem to have all the staining and cultural characteristics of the virulent bacilli, and yet are non-virulent in the sense that they produce no specific toxin. To one who is accustomed to the Loeffler stain it gives as much information as any other, as to the specific virulence of the bacilli. The Neisser stain as well as some others will undoubtedly cause the examiner to suspect more strongly some bacilli of being virulent than the Loeffler stain, but with the varieties met with in New York this suspicion is as apt to be wrong as right. As will be stated more fully later, nothing but animal inoculations with control injections of antitoxin will separate bacilli capable of producing diphtheria toxin from others.

The morphology of the diphtheria bacillus varies considerably with different culture media employed. On glycerin agar or simple nutrient agar there are two distinct types. One grows as smaller and, as a rule, more regular forms than when grown on serum culture media (Fig. 78). The other type shows many thick, Indian-clubbed forms with a moderate number of segments. Short, spindle, lancet, or club-shaped forms, staining uniformly, are all observed. The bacilli which have developed in the pseudomembranes or exudate in cases of diphtheria resemble in shape young bacilli grown on blood serum, but stain more evenly.

Biology.—The Klebs-Loeffler bacillus is non-motile and non-liquefying. It is *aërobic.* It grows most readily in the presence of oxygen,

but also without it. It does not form spores. It begins to develop, but grows slowly at a temperature of 20° C., or even less. It attains its maximum development at 37° C. In old cultures in fluid media, Williams has observed fusion of one bacillus with another. The fused forms live the longest.

Resistance to Heat, Drying, and Chemicals.—Its thermal death point with ten minutes' exposure is about 60° C. Boiling kills in one minute.

FIG. 78

FIG. 79

FIG. 78.—Diphtheria bacilli from agar culture. × 1000 diameters.

FIG. 79.—B. diphtheriæ, No. 81. Forty-eight hours' agar culture. Thick, medium-clubbed rods and moderate number of segments. One year on artificial culture media. × 1410 diameters.

FIG. 80

FIG. 81

FIG. 80.—B. diphtheriæ, No. 57. Forty-eight hours' agar culture. Many segments; long, medium-clubbed ends. One year on artificial media. × 1410 diameters.

FIG. 81.—B. diphtheriæ, S. Twenty-four hours' agar culture. Coccus forms. Segmented granular forms on Loeffler's serum. Only variety found; cases of diphtheria at Children's Home. × 1410 diameters.

It is more easily destroyed by disinfectants than many other bacteria. In the dry state and exposed to diffuse light diphtheria bacilli usually die in a few days or may live for weeks or months; when in the dark, or protected by a film of mucus or albumin, they may live for even longer periods. Thus we found scrapings from a dry bit of membrane to contain vigorous and virulent living bacilli for a period of four months after removal from the throat, and if the membrane had not been at that time completely used, living bacilli could

probably have been obtained for a much longer period. On slate- and lead-pencils, as well as on paper money, they may live for several weeks, while on coins they die in twelve to thirty-six hours. In culture media, when kept at the blood heat, they usually die after a few weeks; but under certain conditions, as when sealed in tubes and protected from heat and light, they retain their virulence for years. The bacillus is not sensitive to cold, for we found it to retain its virulence after exposure for two hours to several hundred degrees below zero.

Growth on Culture Media.—Blood serum, especially coagulated in the form of Loeffler's mixture, is the most favorable medium for the growth of the diphtheria bacillus, and is used particularly for diagnostic purposes in examining cultures from the throats of persons suspected of having diphtheria. For its preparation, see p. 51. If we examine the growth of the diphtheria bacillus in pure culture on blood serum we shall find at the end of from eight to twelve hours small colonies of bacilli, which appear as pearl-gray, whitish-gray, or, more rarely, yellowish-gray, slightly raised points. The colonies when separated from each other may increase in forty-eight hours so that the diameter may be one-eighth of an inch. The borders are usually somewhat uneven. The colonies lying together become confluent and fuse into one mass when the serum is moist. During the first twelve hours the colonies of the diphtheria bacilli are about equal in size to those of the other pathogenic bacteria which are often present in the throat; but after this time the diphtheria colonies become larger than those of the streptococci and smaller than those of the staphylococci. The diphtheria bacilli in their growth never liquefy the blood serum.

FIG. 82

Colonies of diphtheria bacilli. × 200 diameters.

Growth on Agar.—On 1 per cent. slightly alkaline, plain nutrient or glycerin-agar the growth of the diphtheria bacillus is less certain and luxuriant than upon blood serum; but the appearance of the colonies when examined under a low-power lens, though very variable, is often far more characteristic. (See Fig. 82, and Fig. 44, page 62.) For this reason nutrient agar in Petri dishes is used to obtain diphtheria bacilli in pure culture. The diphtheria bacillus obtained from cultures which have developed for some time on culture media grows well, or fairly well, on suitable nutrient agar, but when fresh from pseudomembranes one prevalent type of bacilli grows on these media with great difficulty, and the colonies develop so slowly as to be frequently covered up by the more luxuriant growth of other bacteria when present, or fail to develop at all.

If the colonies develop deep in the substance of the agar they are usually round or oval, and, as a rule, present no extensions; but if near the surface, commonly from one, but sometimes from both sides, they spread out an apron-like extension, which exceeds in surface area the rest of the colony. When the colonies develop entirely on the surface they are more or less coarsely granular, and usually have a dark centre and vary very much in their thickness. The colonies from some are almost translucent; others are thick and almost as luxuriant as the staphylococcus. The edges are sometimes jagged, and frequently shade off into a delicate lace-like fringe; at other times the margins are more even and the colonies are nearly circular. With a high-power lens the edges show sprouting bacilli. The colonies are gray or grayish-white by reflected light, and pure gray with an olive tint by transmitted light.

The growth of the diphtheria bacillus upon agar presents certain peculiarities which are of practical importance. If a large number of the bacilli from a recent culture are implanted upon a properly prepared agar plate a certain and fairly vigorous growth will always take place. If, however, the agar is inoculated with an exudate from the throat, which contains but few bacilli, no growth whatever may occur, while the tubes of coagulated blood serum inoculated with the same exudate contain the bacilli abundantly. Because of the uncertainty, therefore, of obtaining a growth by the inoculation of agar with bacilli unaccustomed to this medium, agar is not a reliable medium for use in primary cultures for diagnostic purposes. A mixture composed of two parts of a 1.5 per cent. nutrient agar and one part of sterile ascitic fluid makes a medium upon which the bacillus grows much more luxuriantly, but not so characteristically. The mixture is made by adding ascitic fluid, warmed to about 45° to 50° C., to the tubes containing the melted agar cooled to 60° C. After shaking, the Petri plates are filled.

Isolation of the Diphtheria Bacillus from Plate Cultures.—Nutrient plain or glycerin-agar is the medium employed to get by plating methods a pure culture from the original serum tube. The agar should be freshly melted and poured in the Petri dish for this purpose. After it has hardened, the layers in a number of plates are streaked across with bacteria from colonies on the serum culture, which appear in size and color like the diphtheria bacilli. Other plates are made from a general mixture of all the bacteria, selected, as a rule, from the drier portion of the serum. Other plates are inoculated from the pellicle of an ascitic broth culture. The plates are left in the incubator for about sixteen hours at 37° C. In the examination of the plates one should first seek for typical colonies, and then later for any that look most nearly like the characteristic picture. Diphtheria colonies are very apt to be found at the edges of the streaks of bacterial growth.

GROWTH IN BOUILLON.—The diphtheria bacilli from about one-half the cultures grow readily in broth slightly alkaline to litmus; the other cultures grow very feebly. The characteristic growth in neutral bouillon is one showing fine grains. These deposit along the sides and bottom of the tube, leaving the broth nearly clear. A few cultures in

neutral bouillon and many in alkaline bouillon produce for twenty-four or forty-eight hours a more or less diffuse cloudiness, and frequently a film forms over the surface of the broth. On shaking the tube this film breaks up and slowly sinks to the bottom. This film is apt to develop during the growth of cultures which have long been cultivated in bouillon, and, indeed, after a time the entire development may appear on the surface in the form of a friable pellicle. The diphtheria bacillus in its growth causes a fermentation of meat-sugars and glucose, and thus if these are present changes the reaction of the bouillon, rendering it distinctly less alkaline within forty-eight hours, and then, after a variable time, when all the fermentable sugars have been decomposed, more alkaline again through the progressing fermentation of other substances. Among the products formed by its growth is the diphtheria toxin.

GROWTH IN ASCITIC OR SERUM BOUILLON.—Diphtheria bacilli grow well in this medium, even when first removed from the throat. They almost always form a slight pellicle at the end of twenty-four to forty-eight hours. To the nutrient bouillon 25 per cent. ascitic fluid or blood serum is added. This culture medium is, as pointed out by Williams, of the greatest value in attempts to get pure cultures of the diphtheria bacillus from solidified serum cultures containing few bacilli.

GROWTH ON GELATIN.—The growth on this medium is much slower, more scanty, and less characteristic than that on the other media mentioned, on account of the lower temperature at which it must be used.

GROWTH IN MILK.—The diphtheria bacillus grows readily in milk, beginning to develop at a comparatively low temperature (20° C.). Thus, milk having become inoculated with the bacillus from some cases of diphtheria may, under certain conditions, be the means of conveying infection to previously healthy persons. The milk remains unchanged in appearance.

Pathogenesis.—The diphtheria bacillus is pathogenic for guinea-pigs, rabbits, chickens, pigeons, small birds, and cats; also in a lesser degree for dogs, goats, cattle, and horses, but hardly at all for rats and mice. In spite of its pathogenic qualities for these animals true diphtheria occurs in them with extreme rarity. As a rule, supposed diphtheritic inflammations in them are due to other bacteria which cannot produce the disease in man.

The virulence of diphtheria bacilli from different sources, as measured by their toxin production in bouillon, varies enormously, but in ascitic fluid it is more alike. Thus 0.002 c.c. of a forty-hour bouillon culture of one bacillus will kill a guinea-pig, which it would require 1 c.c. of the culture of another bacillus to kill. This difference frequently depends on the unequal growth of the bacilli; one culture having fifty times as many bacilli as the other. The same marked variation occurs in the amount of toxin produced by different bacilli in their growth outside of the body. Moreover, the diphtheria bacilli differ greatly in the tenacity with which they retain their virulence when grown outside the body. The bacillus that we have used in the labora-

tory of the Board of Health has retained its virulence almost unaltered for ten years in bouillon cultures. Other bacilli have lost 50 per cent. of their virulence after being kept only a few months. The passage of diphtheria bacilli through the bodies of susceptible animals does not increase their toxin production to any considerable extent.

At the autopsy of animals dying from the poisons produced by the bacilli, the characteristic lesions described by Loeffler are found. At the seat of inoculation there is a grayish focus surrounded by an area of congestion; the subcutaneous tissues for some distance around are œdematous; the adjacent lymph nodes are swollen; and the serous cavities, especially the pleura and the pericardium, frequently contain an excess of fluid, usually clear, but at times turbid; the lungs are generally congested. In the organs are found numerous smaller and larger masses of necrotic cells, which are permeated by leukocytes. The heart and certain voluntary muscular fibres and tissues of nerves usually show degenerative changes. Occasionally there is fatty degeneration of the liver and kidneys. The number of leukocytes in the blood is increased. From the area surrounding the point of inoculation virulent bacilli may be obtained, but in the internal organs they are only occasionally found, unless an enormous number of bacilli have been injected. Paralysis, commencing usually in the posterior extremities and then gradually extending to the whole body and causing death by paralysis of the heart or respiration, is also produced in many cases in which the inoculated animals do not succumb to a too rapid intoxication. In a number of animals we have seen recovery take place three to six weeks after the onset of the paralysis. The occurrence of these paralyses, following the introduction of the diphtheria bacilli, completes the resemblance of the experimental disease to the natural malady in man.

Diphtheria Toxin.—It is evident that a micro-organism which, when injected subcutaneously, destroys the life of susceptible animals and produces such marked anatomical changes in the internal organs, while it is found only at or near the point of inoculation, must owe its pathogenic power to the formation of a poison which, being absorbed, gives rise to toxæmia and death. This poison or *toxin* has been partially isolated by Roux and Yersin, and others, by filtration through porous porcelain from cultures of the living bacilli. It has not yet been successfully analyzed, so that its chemical composition is unknown, but it has many of the properties of proteid substances, and can well be designated by the term active proteid. The poison produced is probably composed of a mixture of several nearly related toxins. Diphtheria toxin is totally destroyed by boiling for five minutes, and loses some 95 per cent. of its strength when exposed to 75° C. for the same time; 73° C. destroys only about 85 per cent. and 60° very little. Lower temperatures only alter it very gradually. Kept from light and air and in cold storage it deteriorates very slowly. The views of Ehrlich and Madsen as to the nature of toxins will be considered in the chapter under its relations to antitoxin.

The Production of Toxin in Culture Media.—The artificial production of toxin in cultures of the diphtheria bacillus has been found to depend upon definite conditions, which are of practical importance in obtaining toxin for the inoculation of horses, and also of theoretical interest in explaining why cases of apparently equal local severity have such different degrees of toxic absorption. The researches of Roux and Yersin laid the foundation of our knowledge. Their investigations have been continued by Theobald Smith, Spronck, ourselves, and others. After an extensive series of investigations we (Park and Williams) came to the following conclusions: Toxin is produced by fully virulent diphtheria bacilli at all times during their life when the conditions are favorable. Under less favorable conditions some bacilli are able to produce toxin while others are not; or it may be that some conditions favor some bacilli while they are deleterious to others. Diphtheria bacilli may find conditions suitable for luxuriant growth, but unsuitable for the production of toxin. The requisite conditions for good development of toxin, as judged by the behavior of a number of cultures, are a temperature from about 35° to 36° C., a suitable culture medium, such as a 2 per cent. peptone nutrient bouillon of an alkalinity which should be about 8 c.c. of normal soda solution per litre above the neutral point to litmus, and prepared from a suitable peptone (Witte) and meat. The culture fluid should be in comparatively thin layers and in large-necked Erlenmeyer flasks, so as to allow of a free access of air. The greatest accumulation of toxin in bouillon is after a duration of growth of the culture of from five to ten days, according to the peculiarities of the culture employed. At a too early period toxin has not sufficiently accumulated; at a too late period it has begun to degenerate. In our experience the amount of muscle-sugar present in the meat makes no appreciable difference in the toxin produced when a vigorously growing bacillus is used, so long as the bouillon has been made sufficiently alkaline to prevent the acid produced by the fermentation of the sugar from producing in the bouillon an acidity sufficient to inhibit the growth of the bacilli. If the sugar does interfere this can be prevented by its previous destruction through the fermentation caused by the growth of the colon bacilli. After the fermentation 0.1 per cent. of glucose should be added. Besides the sugar and allied bodies in the meat there are other substances whose nature is unknown, which hinder or aid a full growth of the bacilli or production of toxin. This is true of bouillon made directly from fresh meat, fermented meat, or meat extracts. With the meat as we obtain it in New York, we get better results with unfermented meat than with fermented. In Boston, with the same bacillus, Smith gets more toxin from the fermented bouillon. Instead of colon bacilli, yeast may be added to the soaking meat, which is allowed to stand at about 25° C.

Under the best conditions we can devise, toxin begins to be produced by bacilli from some cultures when freshly sown in bouillon some time during the first twenty-four hours; from other cultures, for reasons not well understood, not for from two to four days. In neutral bouillon

the culture fluid frequently becomes slightly acid and toxin production may be delayed for from one to three weeks. The greatest accumulation of toxin is on the fourth day, on the average, after the rapid production of toxin has commenced. After that time the number of living bacilli rapidly diminishes in the culture, and the conditions for those remaining alive are not suitable for the rapid production of toxin. As the toxin is not stable, the deterioration taking place in the toxin already produced is greater than the amount of new toxin still forming.

Bacilli, when repeatedly transplanted from bouillon to bouillon, gradually come to grow on the surface only. This characteristic seems to aid in the development of toxin.

The relations of toxin to antitoxin will be considered later in this chapter.

Diphtheria-like Bacilli Not Producing Diphtheria Toxin.—In the tests of the bacilli obtained from hundreds of cases of suspected diphtheria which have been carried out during the past ten years in the laboratories of the Health Department of New York City, in over 95 per cent. of cases the bacilli derived from exudates or pseudomembranes and possessing the characteristics of the Loeffler bacilli have been found to be virulent, that is, producers of diphtheria toxin. But there are, however, in inflamed throats as well as in healthy throats, either alone or associated with the virulent bacilli, occasionally bacilli which, though morphologically and in their behavior on culture media identical with the Klebs-Loeffler bacillus, are yet producers, at least in artificial culture media and the usual test animals, of no diphtheria toxin. Between bacilli which produce a great deal of toxin and those which produce none we find a few of minor grades of virulence. We believe, therefore, in accordance with Roux and Yersin these non-virulent bacilli should be considered as possibly attenuated varieties of the diphtheria bacillus which have lost their power to produce diphtheria toxin. These observers, and others following them, have shown that the virulent bacilli can be artificially attenuated; but the reverse has not been proven that bacilli which produce no specific toxin have later been found to develop it. In our experience some cultures hold their virulence even when grown at 41° C. for a number of months, while others lose it more quickly. Diphtheria-like bacilli are also found which resemble diphtheria bacilli very closely except in toxin production, but differ in one or more particulars. Both these and the characteristic non-virulent bacilli are found occasionally upon all the mucous membranes, both when inflamed and when apparently normal. From varieties of this sort having been found in a number of cases of the condition known as xerosis conjunctivæ, these bacilli are often called xerosis bacilli. Under this name different observers have placed bacilli identical with the diphtheria bacilli and others differing quite markedly from them.

Bacilli Virulent to Guinea-pigs in Spite of Producing no Diphtheria Toxin.—These bacilli are obtained fairly frequently from normal or slightly inflamed throats and may be only slightly pathogenic in

guinea-pigs, or they may kill, as we have found in a number of instances, in doses of 2 to 5 c.c. subcutaneously or intraperitoneally injected. Animals are not protected by diphtheria antitoxin from the action of these bacilli. At autopsy the bacilli are usually found more or less abundantly in the blood and internal organs. The fact that large injections of antitoxic serum hastens the death of guinea-pigs injected with these bacilli, has given rise to the notion that injections of antitoxin might be dangerous in persons in whose throats these bacilli were present, either as saprophytes or, possibly, as inciters of slight disease. It is not the antitoxin, but the serum, which in large doses injures the vitality of the guinea-pigs and so slightly hastens death. Any serum has the effect. These bacilli were first described by Miss Davis[1] from my laboratory and later by Dr. Alice Hamilton in 1904. In my judgment they present no more reason to avoid giving antitoxin than do the streptococci and influenza bacilli. When pathogenic in man they are usually only feebly so.

Location of Diphtheritic Inflammations and Virulence of Bacilli.—Virulent bacilli produce and are found not only in pseudomembranous inflammations of the fauces, larynx, and nasal cavities, but also occasionally in membranous affections of the skin, vagina, rectum, conjunctiva, nose, and ear (simple membranous rhinitis and otitis media). From the severity of an isolated case the virulence of the bacilli cannot be accurately determined. The most virulent bacillus we have ever found was obtained from a mild case of diphtheria simulating tonsillitis. Another case, however, infected by the bacillus proved to be very severe. In localized epidemics the average severity of the cases probably indicates roughly the virulence of the bacillus causing the infection, as here the individual susceptibility of the different persons infected would, in all likelihood, when taken together, be similar to that of other groups; but even in this instance special conditions of climate, food, or race may influence certain localities. Moreover, the bacteria associated with the diphtheria bacilli, and which are liable to be transmitted with them, may influence the severity of and the complications arising in the cases. It must be remembered that bacilli of like toxic power may differ in their liability to infect. Virulence has thus two distinct meanings when used to describe diphtheria bacilli.

Virulent Bacilli in Healthy Throats.—Fully virulent bacilli have frequently been found in healthy throats of persons who have been brought in direct contact with diphtheria patients or infected clothing without contracting the disease. It is, therefore, apparent that infection in diphtheria, as in other infectious diseases, requires not only the presence of virulent bacilli, but also a susceptibility to the disease, which may be inherited or acquired. Among the predisposing influences which contribute to the production of diphtheritic infection may be mentioned the breathing of foul air and living in overcrowded and ill-ventilated rooms, poor food, certain diseases, more particularly

[1] Medical News, April 29, 1899.

catarrhal inflammations of the mucous membranes, and depressing conditions generally. Under these conditions an infected mucous membrane may become susceptible to disease. In connection with Beebe (1894) I made an examination of the throats of 330 healthy persons who had not come in contact, so far as known, with diphtheria, and we found virulent bacilli in 8 only, 2 of whom later developed the disease. In 24 of the 330 healthy throats non-virulent bacilli or attenuated forms of the diphtheria bacillus were found. Very similar observations have been made by others in many widely separated countries.

Persistence of Diphtheria Bacilli in the Throat.—The continued presence of virulent diphtheria bacilli in the throats of patients who have recovered from the disease, and after the disappearance of the exudate, has been repeatedly demonstrated. Beebe and I found that in 304 of 605 consecutive cases the bacilli disappeared within three days after the disappearance of the pseudomembrane; in 176 cases they persisted for seven days, in 64 cases for twelve days, in 36 cases for fifteen days, in 12 cases for three weeks, in 4 cases for four weeks, and in 2 cases for nine weeks. Since then I have met with a case in which they persisted with full virulence for eight months. Later figures agree substantially with these.

Pseudodiphtheria Bacilli.—Besides the typical bacilli which produce diphtheria toxin and those which do not, but which, so far as we can determine, are otherwise identical with the Loeffler bacillus, there are other bacilli found in positions similar to those in which diphtheria bacilli abound, which, though resembling these organisms in many particulars, yet differ from them as a class in others equally important. The variety most prevalent is rather short, plump, and more uniform in size and shape than the true Loeffler bacillus (Fig. 83). On blood-serum their colony growth is very similar to that of the diphtheria bacilli. The great majority of them in any culture show no polar granules when stained by the Neisser method, and stain evenly throughout with the alkaline methylene-blue solution. They do not produce acid by the fermentation of glucose, as do all known virulent and many non-virulent diphtheria bacilli; therefore, there is no increase in acidity in the bouillon in which they are grown during the first twenty-four hours from the fermentation of the meat-sugar regularly present. They are found in varying abundance in different localities in New York City, in about 1 per cent. of the normal throat and nasal secretions, and seem to have now at least no connection with diphtheria; whether they were originally derived from diphtheria bacillus is doubtful; they certainly seem to have no connection with it now. They never produce diphtheria toxin, and to them properly has been applied the name *pseudodiphtheria bacilli*. In bouillon they grow, as a rule, less luxuri-

Fig 83

Pseudodiphtheria bacilli

antly than the diphtheria bacilli. Some of the varieties of the pseudodiphtheria bacilli are as long as the shorter forms of the virulent bacilli. When these are found in cultures from cases of suspected diphtheria they may lead to an incorrect diagnosis. The Neisser staining method is of value here, but, unfortunately, the absence of the stained bodies in smears of mixed cultures is not a sufficient ground to exclude the possibility of their being true diphtheria bacilli. There are also some varieties which resemble the short pseudobacilli in form and staining, but which produce acid in glucose bouillon. These bacilli are found occasionally in all countries where search has been made for them. It may be added here that no facts have come to light which indicate that bacilli which do not produce diphtheria toxin in animals ever produce it in man. It must also be borne in mind, however, that such proof is necessarily very difficult to obtain.

Mixed Infection in Diphtheria.—Virulent diphtheria bacilli, however, are not the only bacteria present in human diphtheria. Various cocci, more particularly streptococci, staphylococci, and pneumococci, are also found associated with Loeffler's bacilli in diphtheria, playing an important part in the disease and leading often to serious complications (sepsis and bronchopneumonia). Indeed, the prognosis in a case of diphtheria is now judged to be graver, other things being equal, according to the degree to which other pathogenic bacteria influence the course of the disease. These cases of so-called *mixed infection* in diphtheria have within recent years attracted considerable attention, and have been the subject of a number of animal experiments. Though the results of these investigations so far have been somewhat indefinite, they would seem to indicate that when other bacteria are associated with the diphtheria bacilli they mutually assist one another in their attacks upon the mucous membrane, the streptococcus being particularly active in this respect, often opening the way for the invasion of the Loeffler bacillus into the deeper tissues or supplying needed conditions for the development of its toxin. Thus diphtheria is not always a primary, but often a secondary disease, following some other infection, as measles or scarlet fever. In most fatal cases of bronchopneumonia following laryngeal diphtheria we find not only abundant pneumococci or streptococci in the inflamed lung areas, but also in the blood and tissues of the organs. As these septic infections due to the pyogenic cocci are in no way influenced by the diphtheria antitoxin, they frequently are the cause of the fatal termination. Other bacteria cause putrefactive changes in the exudate, producing alterations in color and offensive odors.

Pseudomembranous Exudative Inflammations Due to Bacteria other than the Diphtheria Bacilli.—The diphtheria bacillus, though the most usual, is not the only micro-organism that is capable of producing pseudomembranous inflammations. There are numerous bacteria present almost constantly in the throat secretions, which, under certain conditions, can cause local lesions very similar to those in the less-marked cases of true diphtheria. The streptococcus and pneumo-

coccus are the two forms most frequently found in these cases, but there are also others which, under suitable conditions, take an active part in producing this form of inflammation. Among these is a long, slender bacillus which is occasionally found in great abundance in the middle layers of pseudomembranes when the diphtheria bacillus is absent. This bacillus was first described by Vincent.[1] It does not grow readily on artificial media and is not pathogenic in animals. From its presence in the ulcerated processes and false membrane of a number of cases, it is believed to have some causal relation to them. This bacillus does not grow on the serum media, so that the diagnosis must be made from smears.

These cases show many of the local appearances of true diphtheria, the superficial necrosis of the epithelium, the membrane of the glandular swellings. The pseudomembranes may persist for from one to two weeks, or even, in exceptional cases, longer. This bacillus is apparently frequently present in the normal throat, and is probably only able under certain favorable conditions, such as the influence of syphilis, to produce lesions. Nerve degeneration does not follow an attack.

The pseudomembranous angina accompanying scarlet fever, and to a less extent other diseases, may not show the presence of diphtheria bacilli, but only the pyogenic cocci, especially streptococci, or, more rarely, some varieties of little-known bacilli. The deposit covering the inflamed tissues in these non-specific cases is, it is true, usually but not always, rather an exudate than a true pseudomembrane. The majority of these cases, however, are mild affections, being only of importance in adding to the severity of the disease which they complicate. An exception should be made when the larynx is affected, as here the lungs are often secondarily involved. The bacteria which occur in *false diphtheria* are streptococci, staphylococci, diplococci, and sometimes pseudodiphtheria bacilli or bacilli which are morphologically and culturally distinct from the diphtheria bacilli.

Persistence of Varieties of the Bacillus Diphtheriæ and of Diphtheria-like Bacilli.—The fact that there are many varieties of the diphtheria and diphtheria-like bacillus has, we think, been fully established.

But that such varieties are true sub-species with constant characteristics, one variety not changing into another of the established forms, has not been generally accepted. On the contrary, of late the idea seems to be gaining ground among some investigators that all of the various forms of diphtheria-like bacilli are the result of more or less transitory variations of the same species, and hence that the virulent forms are the result of a rapid adaptation to environment and consequent pathogenesis of the non-virulent forms, both typical and atypical.

This question of the relationship of the specifically virulent diphtheria bacillus to non-virulent, diphtheria-like bacilli has been discussed since 1887. It is certainly theoretically possible that the non-virulent forms have been derived from virulent forms. Whether or

[1] Annales de l'Institut Pasteur, August, 1899.

not this is true is an interesting problem for discussion, but has little practical importance. The possibility of the non-toxin producing forms readily assuming their power to produce toxin is of the greatest importance, and if true would cause us to change our present methods of trying to prevent the spread of diphtheria.

Until 1896 no one had brought forward evidence to show that fully non-virulent forms could be made virulent. In this year Trump[1] states that he converted a non-virulent acid, producing bacillus into one capable of killing guinea-pigs with all the symptoms of true diphtheria, by successive passages through guinea-pigs plus a non-fatal dose of diphtheria toxin. Hewlett and Knight[2] state (1897) that they changed a typical virulent diphtheria bacillus into a non-virulent bacillus of the pseudo type by heating for seventeen hours at 45° C. They only succeeded with one culture, though they tried others. They say also that they changed a non-acid pseudodiphtheria bacillus into a typical virulent diphtheria bacillus by culture and passage through guinea-pigs. They obtained similar but not such marked results with other cultures.

Richmond and Salter[3] (1898) and Salter[4] (1899) state that they have changed five pseudodiphtheria bacilli into typical diphtheria bacilli specifically virulent for guinea-pigs by passage through a number of goldfinches.

Bergey[5] was not able to give virulence to non-virulent forms, neither did he find that these latter gave immunity against the former; for these reasons he considers them distinct members of a large group of bacilli at the head of which stands the diphtheria bacillus.

In the work of Wesbrook, Wilson, and McDaniel,[6] on *Varieties of Bacillus Diphtheriæ*, the study is based upon the morphology of the individual bacillus found in smears of throat cultures and pure cultures. They give as a reason for the study of the individual bacillus that in "pure cultures in most instances, especially where they have been derived from typical clinical cases of diphtheria, it is the exception to get even a moderate degree of uniformity in the general shape, size, staining reactions, etc., of the individual bacilli; whilst to get complete uniformity is not to be hoped for," and therefore each culture is probably a mixture of several varieties having been derived from several parents. This seems to us to be probably an erroneous conclusion. They make a provisional classification based upon the morphology of the individual bacilli, into three groups, called granular, barred, and solid, two of the groups into seven types and the other into five, two of the types corresponding with those in the other groups not having been seen. In a study of the types found in the smears from a series of direct cultures derived from clinical cases of diphtheria the

[1] Centralblatt für Bakt., etc., 1896, Band xx. p. 721.
[2] Trans. of the Brit. Inst. of Prev. Med., 1897, 1st series.
[3] Guy's Hospital Reports, 1898.
[4] Trans. of the Jenner Inst. of Prev. Med., 1899.
[5] Pub. of the Univ. of Penn., 1898, new series, No. 4 (other references).
[6] Transactions of the Association of American Physicians, 1900.

authors state that there is generally a sequence of types in the variations which appear throughout the course of the disease, the granular types being the most predominating at the outset of the disease, and these giving place wholly or in part to the barred and solid types shortly before the disappearance of diphtheria-like organisms.

The inference drawn from this work is that the diphtheria bacillus may be rather easily, especially in the throat, converted into non-granular, solidly staining forms of the "pseudodiphtheria" type, and that the converse may occur, and that therefore all diphtheria-like bacilli must be considered a possible source of danger.

Cobbett[1] considers the pseudodiphtheria bacillus as perfectly innocuous to man, but that the relation between the pseudodiphtheria and the diphtheria bacillus remains undecided. He did not meet with bacilli of low virulence. He found a few non-virulent and the others were all highly virulent. He thinks that the reason why the pseudodiphtheria bacilli appear so infrequently during the acute stage is that they are overlooked then because one discovers the virulent bacilli so easily and does not trouble to look any more, and they are found more easily later because the diphtheria bacilli are disappearing and are hard to find; consequently a long and careful search is made, and the pseudodiphtheria bacilli are seen for the first time.

All of this work (including the reports of observers not mentioned in this paper) in regard to the relationship of the different diphtheria-like bacilli to the true diphtheria bacillus may be summed up and tabulated as follows:

Statements in favor of the belief that one form may be changed readily into another.	Statements opposed to this belief.
1. The morphological and cultural characteristics of all diphtheria-like organisms from pseudo to typical virulent forms have some points of resemblance.	The morphological and cultural characteristics of varieties have many points of difference.
2. Diphtheria bacilli possess many grades of virulence from the fully virulent to the non-virulent.	Intermediate grades of virulence are rare.
3. Non-virulent bacilli, both typical and non-typical, have been found more frequently in the convalescing stage of diphtheria than in the acute stage.	There are other reasons than that of change of one form to another to account for this.
4. Non-virulent, atypical bacilli have been the only diphtheria-like organisms found in light anginas.	Virulent diphtheria bacilli have also been frequently found.
5. A sequence of forms in the course of diphtheria and in successive generations of pure cultures, from granular through barred to solid forms, and the converse, has been observed.	The observation is correct only for the forms in the original mixed cultures and is due to the effect of the other bacteria on the development of the diphtheria bacilli or because both varieties were present at the start.

[1] Journal of Hygiene, 1901.

Statements in favor of the belief that one form may be changed readily into another.	Statements opposed to this belief.
6. Solid forms, approaching the atypical non-virulent forms, have been found to be specifically virulent.	Among large numbers of virulent diphtheria no cultures have been found which developed only solid varieties.
7. The virulence of the diphtheria bacillus has been decreased artificially with a change in form and cultural characters, and slightly virulent diphtheria bacilli have been made more virulent.	Artificial decrease of virulence of the diphtheria bacillus has not been accomplished easily, neither have slightly virulent bacilli been made highly virulent.
8. Non-virulent atypical bacilli have, in a few hands, been changed to typical, specifically virulent diphtheria bacilli.	Non-virulent atypical bacilli experimented upon by most observers have retained their characteristics on various artificial culture media under different conditions and in passage through animals.
9. Virulent typical diphtheria bacilli have been apparently changed to solidly staining, non-virulent, diphtheria-like bacilli.	Virulent diphtheria bacilli usually retain their characteristics on artificial culture media under different conditions.

The central idea in the statements of those who believe that diphtheria-like bacilli are simply transitory variations of the species bacillus diphtheriæ is that both the diphtheria bacillus and those bacilli which resemble them have many unstable properties, their form, their cultural characteristics, their pathogenicity all varying within a wide limit, so that one form may assume readily the properties of another form.

The separatists, on the other hand, have found that certain forms possess such stable properties that one is not converted into another, and hence they regard them as distinct species.

In order to make a thorough test of this whole matter Dr. A. W. Williams, of the Research Laboratory, undertook a careful investigation of the subject.

An outline of the work attempted shows the thoroughness of the tests:

1. A study of the diphtheria and diphtheria-like bacilli found in a series of clinically typical diphtherias at the Hospital for Contagious Diseases.

(*a*) Serial smears of cultures directly from throats and noses.

(*b*) Pure cultures isolated from these cultures.

2. A study of the diphtheria and diphtheria-like bacilli found in healthy and diseased throats in a town during an epidemic of diphtheria.

(*a*) Smears of cultures directly from throats.

(*b*) Pure cultures isolated from these cultures.

3. A study of diphtheria and diphtheria-like bacilli found in sore throats during an epidemic of diphtheria at a home for destitute children.

(*a*) Pure cultures.

4. A study of pure cultures of diphtheria and diphtheria-like bacilli from sources other than those given above.

(*a*) On various artificial culture media grown under various conditions.

(*b*) In living tissues of guinea-pigs, white rats, and goldfinches.

(*c*) In symbiosis with other bacteria.

The conclusions reached were as follows: Though some cultures change on some of the media, each changes in its own way, and each culture still has its distinct individuality. After many culture generations, especially when transplanted at short intervals, the different varieties tend to approach each other or rather to run in lines parallel with a common norm, which seems to be a medium-sized, non-segmented bacillus producing granules in early cultures on serum and growing well on all of the ordinary culture media. The non-virulent morphologically typical bacilli must be classed with the virulent varieties as one species, though there is little doubt that more minute study would show distinct species in this group. The atypical pseudo forms, however, which show no tendency to approach the norm of the typical forms, must be classed as distinct species. All of the pseudo and non-virulent morphologically typical varieties when inoculated into the peritoneum of guinea-pigs in immense doses cause death. Attempts have been made to give more virulence to some of these varieties by successive peritoneal inoculations, but in no instance has any increase of virulence or decided change in morphological or cultural characteristics been noted. Two of the non-virulent, morphologically typical varieties have also been grown in symbiosis with virulent streptococci in broth for ninety culture generations transplanted every three to four days, but when separated no change in virulence or other characteristics was noted. Two other varieties of non-virulent morphologically typical bacilli have been inoculated into goldfinches with no result. In large doses they appear to be perfectly innocuous to these birds as well as do four varieties of pseudobacilli, contrary to the results of Richmond and Salter.

Since there are so many different forms or varieties of diphtheria-like bacilli, it is quite possible that some of them are derived from strains of the diphtheria bacillus and that under certain conditions they readily regain its characteristics. This seems to be the only way to explain the apparent discrepancies in the results obtained by different observers. Such closely related varieties, however, do not appear to be common in New York City at the present time. So we may safely say that in this region at least non-virulent diphtheria-like organisms retain their characteristics under various artificial and natural conditions, and that they may be regarded from a public health standpoint as harmless. These studies seem to demonstrate that the morphologically typical diphtheria bacillus is a distinct species from the atypical diphtheria-like bacilli and so-called pseudo forms, and that it has many true morphological varieties or sub-species which, while showing transitory ontogenic variations due to change in environment and life habit, have more or less per-

sistent phylogenic characteristics which reappear when the organism is placed in a previous environment.

Transmission of Diphtheria.—The possibility of the transmission of diphtheria from animals to man cannot be disputed; we have met with one instance where a cat had malignant diphtheria, and many other animals can be infected, but there are no authentic cases of such transmission on record. So-called diphtheritic disease in animals and birds is usually due to other micro-organisms than the diphtheria bacilli.

Let us consider some of the means by which the disease may be communicated. In actual experiment the bacilli have been observed to remain virulent in bits of dried membrane for twenty weeks. Dried on silk threads Abel reports that they may sometimes live one hundred and seventy-two days, and upon a child's plaything which had been kept in a dark place they lived for five months. The virulent bacilli have been found on soiled bedding or clothing of a diphtheria patient, or drinking-cups, candy, shoes, hair, slate-pencils, etc. Besides these sources of infection by which the disease may be indirectly transmitted, virulent bacilli may be directly received from the pseudomembrane, exudate, or discharges of diphtheria patients; from the secretions of the nose and throat of convalescent cases of diphtheria in which the virulent bacilli persists; and from the healthy throats of individuals who acquired the bacilli from being in contact with others having virulent germs on their persons or clothing. In such cases the bacilli may sometimes live and develop for days or weeks in the throat without causing any lesion. When we consider that it is only the severe types of diphtheria that remain isolated during their actual illness, the wonder is not that so many, but that so few, persons contract the disease. It indicates that very frequently virulent bacilli are received into the mouth, and then either find no condition there suitable for their growth or are swept away by food or drink before they could effect a lodgement.

Susceptibility to and Immunity against Diphtheria.—An individual susceptibility, both general and local, to diphtheria, as in all infectious diseases, is necessary to contract the disease. Age has long been recognized to be an important factor in diphtheria. Children within the first six months of life are but little susceptible, the greatest degree of susceptibility being between the third and the tenth year, while adults are almost immune.

As the result of animal experiments, it is now known that an artificial immunity against diphtheria can be produced, at least for a considerable length of time, by the development of substances directly antidotal to the diphtheria toxin. By the inoculation of virulent or somewhat attenuated cultures or of diphtheria toxin, Fraenkel, Behring, Wernicke, Aronson, Roux, and since then many others, have succeeded in immunizing animals; but the most important and valuable results are those which have been obtained by Behring, in conjunction with others, who showed that the blood of immune animals contains a substance which neutralizes the diphtheria toxin. The blood serum of persons who have recovered from diphtheria has been found also to possess

this protective property, which it acquires about a week after the beginning of the disease, and loses again in a few weeks or months. Moreover, the blood serum of many individuals, usually adults, who have never had diphtheria often has a slight general antitoxic property.

Antitoxic Serum.—The knowledge derived from these remarkable investigations into the protective powers of the blood serum of immunized animals has been employed with the most brilliant results for the prevention and early treatment of diphtheria in man. The discovery of the method of the production of antitoxic serum or antitoxin in animals, and its practical application to the treatment and cure of diphtheria, has been shared by many experimenters, at first chiefly in Germany and France, and later in this country.

Results of the Antitoxin Treatment of Diphtheria.—The conclusions arrived at by Biggs and Guerard, after a review of all the statistics and opinions published since the beginning of the antitoxin treatment in 1892, were as follows:

"It matters not from what point of view the subject is regarded, if the evidence now at hand is properly weighed, but one conclusion is or can be reached—whether we consider the percentage of mortality from diphtheria and croup in cities as a whole, or in hospitals, or in private practice; or whether we take the absolute mortality for all the cities of Germany whose population is over 15,000, and all the cities of France whose population is over 20,000; or the absolute mortality for New York City, or for the great hospitals in France, Germany, and Austria; or whether we consider only the most fatal cases of diphtheria, the laryngeal and operative cases; or whether we study the question with relation to the day of the disease on which treatment is commenced, or the age of the patient treated; it matters not how the subject is regarded or how it is turned for the purpose of comparison with previous results, the conclusion reached is always the same—namely, there has been an average reduction of mortality from the use of antitoxin in the treatment of diphtheria of not less than 50 per cent., and under the most favorable conditions a reduction to one-quarter, or even less, of the previous death rate. This has occurred not in one city at one particular time, but in many cities, in different countries, at different seasons of the year, and always in conjunction with the introduction of antitoxin serum and proportionate to the extent of its use." Except where immunization has been practical on a large scale no reduction in the number of cases of diphtheria has been evident.

Production of Diphtheria Antitoxin for Therapeutic Purposes.—As a result of the work of years in the laboratories of the Health Department of New York City, the following may be laid down as a practical method:

A strong diphtheria toxin should be obtained by taking a very virulent culture and growing it in broth under the conditions described on page 194. The culture, after a week's growth, is removed, and having been tested for purity by microscopic and culture tests is rendered sterile by the addition of 10 per cent. of a 5 per cent. solution

of carbolic acid. After forty-eight hours the dead bacilli have settled on the bottom of the jar and the clear fluid above is syphoned off or it is filtered through ordinary sterile filter paper and stored in full bottles in a cold place until needed. Its strength is then tested by giving a series of guinea-pigs carefully measured amounts. Less than 0.01 c.c., when injected hypodermically, should kill a 250-gram guinea-pig.

The horses used should be young, vigorous, of fair size, and absolutely healthy. Vicious habits, such as kicking, etc., make no difference, of course, except to those who handle the animals. The horses are severally injected with an amount of toxin sufficient to kill five thousand guinea-pigs of 250 grams' weight (about 20 c.c. of strong toxin). After from three to five days, so soon as the fever reaction has subsided, a second subcutaneous injection of a slightly larger dose is given. With the first three injections of toxin 10,000 units of antitoxin are given. If antitoxin is not mixed with the first doses of toxin only one-tenth of the doses advised is to be given. At intervals of from five to eight days increasing injections of pure toxin are made, until at the end of two months from ten to twenty times the original amount is given. There is absolutely no way of judging which horses will produce the highest grades of antitoxin. Very roughly, those horses which are extremely sensitive and those which react hardly at all are the poorest, but even here there are exceptions. The only way, therefore, is at the end of six weeks or two months to bleed the horses and test their serum. If only high-grade serum is wanted all horses that give less than 150 units per c.c. are discarded. If moderate grades only are desired, all that yield 100 units may be retained. The retained horses receive steadily increasing doses, the rapidity of the increase and the interval of time between the doses (three days to one week) depending somewhat on the reaction following the injection, an elevation of temperature of more than 3° F. being undesirable. At the end of three months the antitoxic serum of all the horses should contain over 300 units, and in about 10 per cent. as much as 800 units in each cubic centimetre. Very few horses ever give above 1000 units, and none so far has given as much as 2000 units per c.c. The very best horses if pushed to their limit continue to furnish blood containing the maximum amount of antitoxin for several months, and then, in spite of increasing injections of toxin, begin to furnish blood of gradually decreasing strength. If every nine months an interval of three months' freedom from inoculations is given, the best horses furnish high-grade serum during their periods of treatment for from two to four years.

In order to obtain the serum the blood is withdrawn from the jugular vein by means of a sharp-pointed cannula, which is plunged through the vein wall, a slit having been made in the skin. The blood is carried by a sterile rubber tube into large Erlenmeyer flasks and allowed to clot, the flasks, however, being placed in a slanting position before clotting has commenced. The serum is drawn off after four days by means of sterile glass and rubber tubing, and is stored in large flasks.

From this, as needed, small phials are filled. The phials and their stoppers, as indeed all the utensils used for holding the serum, must be absolutely sterile, and every possible precaution must be taken to avoid contamination of the serum. An antiseptic may be added to the serum as a preservative, but it is not necessary except when the serum is to be sent to great distances, where it cannot be kept under supervision.

Kept from access of air and light and in a cold place it is fairly stable, deteriorating not more than 40 per cent., and often much less, within a year. Diphtheria antitoxin, when stored in phials and kept under the above conditions, contains within 10 per cent. of its original strength for at least two months; after that it can be used by allowing for a maximum deterioration of 5 per cent. for each month. The antitoxin in old serum is just the same as in that freshly bottled, only there is less of it. Almost all producers put more units in the phials than the label calls for so as to allow for the gradual loss of strength.

The nature of diphtheria antitoxin has until recently been known almost wholly from its physiological properties. Experiments have seemed to show that it was either closely bound to the serum globulins or was itself a substance of proteid nature closely allied to serum globulin. Mr. J. P. Atkinson, when assistant chemist in the laboratory, found that antitoxic and normal horse serum react similarly toward $MgSO_4$, in that the globulin is precipitated completely from the other constituents of the serum. In the case of antitoxic serum the globulin precipitate carries with it all of the antitoxic power of the serum, leaving the filtrate without any neutralizing power against the diphtheria toxin. When watery solutions of this globulin are saturated with NaCl a precipitate occurs. When the solution is heated a series of further precipitates take place, as follows: Cloudiness appears at 40°, 49°, 57°, and 67° C.; complete precipitation occurs at 45°, 54°, 62°, and 72° C. Each of these precipitates has antitoxic properties, and the total quantities contain all the original antitoxin except ome 5 per cent., which is evidently destroyed by the higher temperatures required for the last two precipitates. After the last precipitate the solution is free of globulin and also of all antitoxic properties.

A further fact developed by Atkinson is that the globulins increase markedly in the serum of horses as the antitoxin strength increases. It seems, therefore, from the above that diphtheria antitoxin has the characteristics of the serum globulins. Antitoxin is destroyed by prolonged moderate heat (60° C.) and by short exposure to higher temperatures (95° to 100° C.). It is less sensitive than diphtheria toxin.

Diphtheria antitoxin has the power of neutralizing diphtheria toxin, so that when a certain amount is injected into an animal before or together with the toxin it overcomes its poisonous action. The facts in favor of a direct action of antitoxins upon their corresponding toxins have recently been briefly summarized by Cobbett as follows:

1. Certain reactions have been observed to take place between these substances outside the animal body (venom, ricin, crotin, tetanus toxin, diphtheria toxin, and their corresponding antitoxins).

2. Various attempts to separate the toxins and antitoxins from neutral mixtures have been failures. Partial successes have, at least in some instances, been shown to depend upon the fact that insufficient time for their complete union was allowed, separation being no longer possible if this were granted.

3. The accuracy of the titration of toxins and antitoxins to within 1 per cent. of error.

4. Neutralization takes place according to the law of multiple proportions, *i. e.*, to save an animal from 1000 fatal doses of diphtheria toxin requires little more than a hundred times as much antitoxin as is required for ten fatal doses, the resistance of the animal itself accounting for the difference.

5. The fact that the potency of antitoxin is greatly increased if it is allowed to come in contact with the toxin outside the animal body; and is increased still further if allowed to remain for sufficient time in contact with the toxin at a suitable temperature. The union takes place more quickly at a warm than at a cold temperature.

The facts now known, therefore, indicate that the antitoxins of tetanus and diphtheria, of snake-poison, of ricin, etc., enter into direct chemical combination with their respective toxins. Many points, however, are still far from clear as to the manner in which both toxins and antitoxins act.

Testing of Antitoxin.—This power, possessed by a definite quantity of antitoxin to neutralize a certain amount of toxin, is utilized in testing antitoxin. Guinea-pigs of about 250 grams' weight are subcutaneously injected with one hundred or with ten fatal doses of a standardized toxin, which have been previously mixed with an amount of antitoxin believed to be sufficient to protect from the toxin. If the guinea-pig lives four days, but dies soon after, the amount of antitoxin added to the one hundred fatal doses of toxin was just 1 unit. If the guinea-pig dies earlier, less than 1 unit was added.

Use of Antitoxin in Treatment and Immunization.—The antitoxin in the higher grades is identical with that in the lower grades; there is simply more of it in each drop of the serum. In treatment, however, for the same amount of antitoxin we have to inject less blood serum with the higher grades, and, therefore, have somewhat less danger of rashes and other deleterious results. The amount of antitoxin required for immunization is 300 units for an infant, 500 for an adult, and proportionately for those between these extremes. After the observation of the use of antitoxin in the immunization of several thousand cases, I have absolute belief in its power to prevent an outbreak of diphtheria for at least two weeks, and also of its almost complete harmlessness in the small doses required. If it is desired to prolong the immunity the antitoxin injection is repeated every two weeks. For treatment, mild cases should be given 1500 units, moderate cases 2000 to 4000 units, and severe cases 5000 units. Where no improvement follows in twelve hours the dose should be repeated. Intravenous injections give most rapid effect. Antitoxin is not absorbed when given by the mouth.

No deleterious effects are to be feared except a rash, with some rise of temperature, in about 20 per cent. of the cases. In about 1 per cent. of the cases swelling and tenderness of one or more joints occur. Except in septic cases no permanent disability follows.

There are on record some five or six cases where following an injection in a case of diphtheria sudden death has followed. The result is probably due to the excitement caused by the operation rather than to the serum.

With the serum from some horses the rashes are very infrequent, while with that from others they occur more often. The same horse will at one time furnish a serum which produces no rashes and at another one which gives a great number. No way has yet been found to eliminate them entirely. Filtering and moderate heating produce little effect. Standing for some months causes a precipitate to occur, and the clear serum seems somewhat less liable to produce rashes than when it was fresh.

Use of a Serum to Eradicate Diphtheria Bacilli from Convalescents and Healthy Persons.—A great difficulty in combating diphtheria is this: that in healthy children, but especially in diphtheria convalescents, despite the use of antitoxin, the diphtheria bacilli often remain in the nasopharynx for a very long time. This is extremely annoying, because a child so affected cannot be sent to school until all diphtheria bacilli have disappeared from the nasopharynx. Wassermann has done as follows: A strongly agglutinating, multipartial diphtheria serum is evaporated to dryness *in vacuo,* mixed with sugar of milk, pulverized, and pressed into tablets. These tablets when dissolved in the mouth cause the fluids in the mouth to become strongly agglutinating. The question was, and is, whether this process of agglutination will help us to get rid of the diphtheria bacilli from the nasopharynx more quickly and surely than was heretofore possible. His clinical experiments thus far made speak in favor of the employment of this serum. Whereas, it is no rarity for diphtheria bacilli to be present in the throats of convalescents for weeks, he found that in the cases in which these tablets have been used the bacilli disappeared within a few days.

His method is this: A tablet is allowed to dissolve in the mouth about every two hours, and then, after fifteen minutes, the child's nasopharynx is rinsed out with an indifferent fluid in the form of a spray or gargle. He conceives the action to be such that, whereas when this serum is not employed the diphtheria bacilli are scattered diffusely throughout the nasopharynx, under the influence of this serum they are agglutinated or clumped together. The diphtheria bacilli are massed together more or less by the serum, and these clumps are then removed by the subsequent rinsing. In this way they are so much decreased in amount that the natural power of the organism is able much more quickly to make away with those remaining. He has hopes that this new diphtheria serum will be destined to be of great service, especially in making prophylaxis easier and in making it possible to send the diphtheria convalescents to school earlier than heretofore.

My own experience with this serum have been few and unsatisfactory.

Development of Agglutinins for Diphtheria Bacilli.—By the injections of the bodies of diphtheria bacilli into animals agglutinins have been developed in sufficient amount to act in 1:5000 dilutions of the serum. The serum produced from diphtheria bacilli does not agglutinate pseudodiphtheria bacilli in high dilutions. The serum of patients convalescent from diphtheria has, as a rule, little agglutinating power. This test is not used in diagnosis.

Persistence of Antitoxin in the Blood.—When injections of toxin are stopped in a horse the antitoxin is slowly eliminated, so that there is a loss of about 20 per cent. a week. In from three to five months all appreciable antitoxin has been eliminated.

Technical Points upon the Testing of Diphtheria Antitoxin and the Relations between the Toxicity and Neutralizing Value of Diphtheria Toxin.—During the earlier investigations the filtered or sterilized bouillon, in which the diphtheria bacillus had grown and produced its "toxin," was supposed to require for its neutralization an amount of antitoxin directly proportional to its toxicity as tested in guinea-pigs. Thus, if from one bouillon culture ten fatal doses of "toxin" were required to neutralize a certain quantity of antitoxin, it was believed that ten fatal doses from every culture, without regard to the way in which it had been produced or preserved, would also neutralize the same amount of antitoxin. Upon this belief was founded the Behring-Ehrlich definition of an antitoxin unit.

The results of tests by different experimenters with the same antitoxic serum, but with different diphtheria toxins, proved this opinion to be incorrect. Ehrlich[1] deserves the credit for first clearly perceiving and publishing this. He obtained from various sources twelve toxins and compared their neutralizing value upon antitoxin; these tests gave most interesting and important information. The results in four toxins, which are representative of the twelve, are as shown in the following table:

Toxin specimen number of Ehrlich.	Estimated "minimal" fatal dose for 250-gm. guinea-pigs.	Smallest number of fatal doses of toxic bouillon required to kill a 250-gm guinea-pig within 5 days, when mixed with one antitoxin unit. "L_+ Ehrlich."	Fatal doses required to "completely neutralize one antitoxin unit" as determined by the health of the guinea-pig remaining unaffected. "L_0" Ehrlich.	$L_+ - L_0$ = fatal doses.	Data upon "toxin" specimen given by Ehrlich.
4	0.009	39.4	33.4	6	Old, deteriorated from 0.003 to 0.009
7	0.0165	76.3	54.4	22	Fresh toxin, preserved with tricresol.
9	0.089	123	108	15	A number of fresh cultures grown at 37° C. four and eight days.
12	0.0025	100	50	50	Tested immediately after its withdrawal.

[1] Die Wertbemessung des Diphtherieheilserums und deren theoretische Grundlagen. Klinisches Jahrbuch, 1897.

From the facts set forth in the table, Ehrlich believed that the diphtheria bacilli in their growth produce a toxin which, so long as it remains chemically unaltered, has a definite poisonous strength with a definite value in neutralizing antitoxin. This neutralization he believed to be a chemical union, in which two hundred fatal doses of toxin for a 250 grams' weight guinea-pig combine with one unit of antitoxin. The toxin is, however, an unstable compound, and begins to change almost immediately into substances which are not, at least acutely, poisonous, but which retain their full power to neutralize antitoxin. These substances, according to Ehrlich, fell into three groups. The first has more affinity for combining with the antitoxin than the toxin itself (protoxoids). The second has the same affinity (syntoxoids). The third has less affinity (epitoxoids). The development of Ehrlich's theories of the chemical nature of this union of pure and modified toxin with antitoxin is described on page 165. The toxin with its haptophore group intact but with its toxophore altered is the toxoid.

According to him, if a mixture of toxoids and toxin is added to antitoxin, the protoxoids first combine with the antitoxin; then the syntoxoids and the toxin combine in equal proportions, so long as the supply lasts, with the amount of antitoxin remaining, or, if there is a surplus, with enough to satisfy them; finally, if any antitoxin remains, the epitoxoids unite with it.

The results of these experiments of Atkinson and myself[1] were fully in accord with those published by Ehrlich as to the varying neutralizing value of a minimal fatal dose of "toxin;" they, however, also indicate roughly a general law in accordance with which these changes occur.

The neutralizing value of a fatal dose of toxin is at its lowest in the culture fluid when the first considerable amounts of toxin have been produced. After a short period, during which the quantity of toxin in the fluid is increasing, the neutralizing value of the fatal dose begins to increase, at first rapidly, then more slowly.

While the culture is still in vigorous growth and new toxin is being produced, the neutralizing value of the fatal dose fluctuates somewhat, but with a generally upward tendency. After the cessation of toxin production the neutralizing value of the fatal dose increases steadily until it becomes five to ten times its original amount.

In our experiments the greatest value for L_+ was 126, the least 27. As at six hours L_+ was only 72 and at twenty-eight hours only 91, we doubt whether L_+ ever reaches above 150.[2] When we seek to analyze the above-described process we find certain facts which seem partly to explain it.

In the fluid holding the living bacilli we have, after the first few

[1] Journal of Experimental Medicine, vol. iii., No. 4.

[2] L_+ = fatal doses of toxin required to kill a guinea-pig in four days after having been mixed with one unit of antitoxin.

L_0 = fatal doses of toxin required to fully neutralize one unit of antitoxin.

hours of toxin formation, a double process going on—one of deterioration in the toxin already accumulated, which tends to increase the neutralizing value of the fatal dose; the other of new toxin formation, which probably tends to diminish the neutralizing value. The chemical changes produced by the growth of the bacilli in the bouillon tend to aid one or the other of these processes, and so to make, from hour to hour, slight changes in the value of the fatal dose. Later, with the period of cessation of toxin production, the gradual deterioration of the toxicity alone continues, and the fatal dose gradually and steadily increases in its neutralizing value.

With greater information Ehrlich has had to modify greatly the details of his explanation of the reason of the variation in the ratio between toxicity and neutralizing value of toxin. He now accepts the fact that diphtheria culture fluid contains at least two toxins.

Partial Saturation Method of Study.—Much additional information concerning the nature of toxin has been gained by experimenting with mixtures of toxin and antitoxin, in which the two are present in varying proportions. This is the "partial saturation" method of Ehrlich. Through a number of experiments Ehrlich obtained information which permitted him to estimate that 200 "binding units" are represented in the amount of diphtheria toxin (hypothetically pure) which is exactly neutralized by one antitoxin unit. If the entire amount of antitoxin—*i. e.*, 200/200, is added to the amount of toxin in question, complete neutralization of the latter, of course, occurs. In case the toxin is entirely pure, 199/200 of the antitoxin unit would destroy all but 1/200 of the initial toxicity; and 150/200, or 100/200, or 75/200, etc., of the antitoxin when added would permit corresponding degrees of toxicity to be demonstrated through animal inoculations. It was found, however, that neutralization according to this simple scale did not take place. The results were complicated, and Ehrlich has found it convenient to express them graphically in the form of the "toxin spectrum." For example, let 199/200 of the antitoxin unit be added to the proper amount of the toxin, 198/200 to another similar amount, 197/200 to another, etc., down to 150/200. In the last mixture, 50 out of the 200 binding units which the toxin possesses are free, and these 50, rather than some other 50, are free because they have less affinity for the antitoxin than the 150 units which were bound. It has been found that those units which first become free are much less toxic than a corresponding amount of the original toxin. It was thought that they might have lost their toxophore groups—*i. e.*, that they were toxoids; and because of their weak affinity for antitoxin they were called epitoxoids. It was found, however, that they possessed a rather constant though low degree of toxicity and that the toxic action was characteristic. Injection was followed by some local œdema, then by a long incubation period, and finally by cachexia and paralysis. On account of this characteristic toxic action and the long incubation period, Ehrlich has concluded that the so-called epitoxoid is in reality a separate toxin secreted by the diphtheria bacillus.

Toxon.—This he now designates as toxon in order to distinguish it from that other constituent of diphtheria bouillon, the toxin, which causes the acute phenomena of diphtheria.

Let one now add still smaller amounts of the antitoxin unit to the 200 binding units of the toxin. When 149/200 are added it is found that a certain amount of true toxin remains free, and, moreover, is free in direct proportion to the amount of antitoxin withheld. Consequently when but 50/200 antitoxin unit is added the amount of free toxin corresponds to 100 binding units. If true toxin only remained a continuation of the experiment would show toxin equals 150. It could then be said that the constitution of this toxin is: toxin 150 and toxon 50. However, it may be found that as 49/200, 48/200, etc., to 0/200 antitoxin unit are added, no increase of free toxin is found, although the antitoxin added has been found. Therefore, the 50 toxin binding units which have the greatest affinity for antitoxin are non-toxic—*i. e.*, they are toxoids, and since they have the maximum affinity for antitoxin they are called protoxoids.

It has been assumed also that a toxoid may exist which has an affinity for antitoxin exactly equalling that which toxin possesses; this as yet purely hypothetical constituent bears the name of syntoxoid.

Refinements in experimentation show that even the true toxin is not uniform in its virulence and its affinity for antitoxin. Accordingly, a prototoxin, a deuterotoxin, and a tritotoxin may be recognized by this same partial saturation method. For example, it may be found that when a portion of the antitoxin unit, between the limits of 149/200 and 125/200, is withheld, a toxin is left free which is less virulent than that remaining free between the limits of 124/200 and 100/200; and from this point on the new unbound toxin may be still more virulent. The first would be tritotoxin, the second deuterotoxin, and the third prototoxin.

A "spectrum" having been worked out for a toxin when fresh, an examination made some time later, a year for example, may show many changes. The prototoxin zone and portions of the deuterotoxin or tritotoxin may also have disappeared because of toxoid formation. These changes have led to the recognition of an alpha and a beta modification of the toxin portions. The alpha modifications of all three toxins readily become toxoids. Only the beta modification of the deuterotoxin remains constant. The toxon portion also remains relatively intact.

Summary.—To summarize Ehrlich's views as to the nature of diphtheria toxin: The diphtheria bacillus secretes two toxins, one of which, the toxin, causes the acute phenomena of diphtheria intoxication, while the other, the toxon, causes cachexia and paralysis after a rather long period of incubation. The non-toxic toxin, or toxoid, appears as the result of the degeneration of the toxophore group of the toxin, the haptophore group remaining intact. The toxin may be separated into three divisions, which vary in their affinity for antitoxin—prototoxin, deuterotoxin, and tritotoxin. On the same basis there are three toxoids —protoxoids, syntoxoids, and epitoxoid (the toxon)—the first having

the greatest affinity for antitoxin, while the epitoxoid has the least. The toxins are divided into an alpha and a beta portion, depending on the ease with which they are changed into toxoids. All of these substances unite with tissue cells and with antitoxin through the agency of a haptophore group, while the toxicity depends on the presence of a toxophore group in the toxin or toxon molecule.

Bordet and others refuse to accept these complicated conceptions of Ehrlich and the whole matter is at the present time under active discussion. Thus the existence or non-existence of toxons has excited a great deal of discussion among investigators. The great Swedish chemist, Arrhenius, has recently given much attention to toxins and is applying the principles of physical chemistry to the study of toxins and antitoxins. It is a well-known fact that some chemical substances when in solution have the power of breaking up into their constituent parts; thus sodium chloride breaks up in part into sodium and chlorine, as sodium or chlorine ions or electrolytes. The dissociated sodium and chlorine may then enter into combination with any other suitable substance which may be present. Arrhenius holds that this is the case with the toxin-antitoxin molecule, that it may to a certain extent again break up into separate toxin and antitoxin. He believes that this dissociated toxin is the substance which Ehrlich has been calling toxon. Madsen, who formerly had done much work with toxons, has now joined with Arrhenius in support of the dissociation theory. In spite of their reasoning Ehrlich and his followers continue to uphold the toxon as an independent toxic substance. Recent investigations throw doubt on both explanations as being at all final.

Standardizing of Antitoxin Testing.—Ehrlich has contributed greatly to uniformity in results in testing antitoxin by calling attention to the necessity of selecting a suitable toxin and by employing and distributing an antitoxin as a standard to test toxins by. In this way smaller testing stations can make their results correspond with those of the central station. The United States Marine Hospital laboratories have recently begun to distribute to laboratories in the United States an equally carefully standardized serum.

In spite of the great variations in the neutralizing value of a fatal dose in different toxins we do not believe that even before adopting the use of a standard serum there has been any such great difference in the toxins used by the different stations for testing purposes. Most laboratories have taken the culture fluid at about the time of its greatest toxicity, and the neutralizing value of a fatal dose of this toxin would seldom vary more than 10 per cent. above or below the standard now adopted in Germany by the government testing station.

Where error has been made it has usually been by taking too old culture fluids, which would cause the antitoxin strength of samples tested to be estimated below and not above its real value. Culture 8, which is used not only by the New York Board of Health Laboratory, but by many other laboratories in the United States and Europe, fortunately produces on the sixth to eighth day—the time at which the

culture is usually removed—a toxin which usually grades Ehrlich's antitoxin within 5 per cent. of the strength given by him.

We believe that by using such a bacillus we can, after gaining a fuller knowledge of its characteristics, obtain a toxin of a known and suitable neutralizing value, and thus always correctly standardize an antitoxic serum in case the present stations ceased to supply a testing serum. This is certainly true for the bacillus which we have used for the past ten years. A preparation of a carefully tested antitoxin is of immense value in ensuring a uniform standard among the different testing stations and in allowing of comparison between them.

The old definition of Behring and Ehrlich, that an antitoxin unit contains the amount of antitoxin which will protect the life of a guinea-pig from one hundred fatal doses of toxin, must be modified so as to be defined as that amount of antitoxin which will neutralize one hundred fatal doses of a toxin similar to that adopted as the standard—namely, one having approximately the characteristics of toxins in cultures at the height of their toxicity.

The actual test of an antitoxin serum is, therefore, carried out as follows: Six guinea-pigs are injected with mixtures of toxin and antitoxin. In each of the mixtures there is 100 times the amount of a toxin such as just described, which will kill 250 grm. of guinea-pig on an average in ninety-six hours. In each of the mixtures the amount of antitoxin varies; for instance, No. 1 would contain 0.002 c.c. serum; No. 2, 0.003 c.c.; No. 3, 0.004 c.c.; No. 4, 0.005 c.c., etc. If at the end of the fourth day Nos. 1, 2, and 3 were dead and Nos. 4, 5, and 6 were alive we would consider the serum to contain 200 units of antitoxin for each cubic centimetre. When we mix only ten fatal doses of toxin with one-tenth of the amount of antitoxin used with one hundred fatal doses the guinea-pig must remain well. The mixed toxin and antitoxin must remain together for fifteen minutes before injecting.

Relation of Bacteriology to Diagnosis.—We believe that all experienced clinicians will agree that, when left to judge solely by the appearance and symptoms of a case, there are certain mild exudative inflammations of the throat which are at times excited by diphtheria bacilli and at times by other bacteria.

It is not meant to imply that a case is one of true diphtheria simply because the diphtheria bacilli are present, but rather that the doubtful cases not only have the diphtheria bacilli in the exudate, but are capable of giving true characteristic diphtheria to others, or later develop it characteristically themselves; and that those in whose throats no diphtheria bacilli exists can under no condition give true characteristic diphtheria to others, or develop it themselves unless they receive a new infection. It is, indeed, true, as a rule, that cases presenting the appearance of ordinary follicular tonsillitis in adults are not due to the diphtheria bacillus. It is also true that now and then a case having this appearance is one of diphtheria, and almost every physician has seen such cases from time to time in households infected with diphtheria. On the other hand, in small children

mild diphtheria very frequently occurs with the semblance of rather severe ordinary follicular tonsillitis, due to the pyogenic cocci, and in large cities where diphtheria is prevalent all such cases must be watched as being more or less suspicious. As showing doubt in our judgment, I think most would feel that if in any case exposure to diphtheria is known to have occurred, even a slightly suspicious sore throat would be regarded as probably due to the diphtheria bacilli. If, on the other hand, no cases of diphtheria have been known to exist in the neighborhood, even cases of a more suspicious nature would probably not be regarded as diphtheria.

Appearances Characteristic of Diphtheria.—The presence of irregular-shaped patches of adherent grayish or yellowish-gray pseudomembrane or some other portions than the tonsils is, as a rule, an indication of the activity of the diphtheria bacilli. Restricted to the tonsils alone their presence is less certain.

Occasionally, in scarlatinal angina or in severe phlegmonous sore throats, patches of exudate may appear on the uvula or borders of the faucial pillars, and still the case may not be due to the diphtheria bacilli; these are, however, exceptional. Thick, grayish pseudomembranes which cover large portions of the tonsils, soft palate, and nostrils are almost invariably the lesions produced by diphtheria bacilli.

The very great majority of cases of pseudomembranous or exudative laryngitis, in the coast cities at least, whether an exudate is present in the pharynx or not, are due to the diphtheria bacilli. Cases in which no exudate is apparent and those in which the laryngeal obstruction is sudden and the spasmodic element is marked are, however, frequently due to the activity of other bacteria. Nearly all membranous affections of the nose are true diphtheria. When the membrane is limited to the nose the symptoms are, as a rule, very slight; but when the nasopharynx is involved the symptoms are usually grave. Ordinarily a small area of inflammation indicates a slight or moderate severity, and an extensive area a severe infection.

Most cases of pseudomembranes and exudates, entirely confined to portions of the tonsils in adults, are not due to the diphtheria bacilli, although a few cases presenting these symptoms are. The more complete the involvement of the tonsils the more apt the case is to be due to them. Cases presenting the appearances found in scarlet fever, in which a thin, grayish membrane lines the borders of the uvula and faucial pillars, are rarely diphtheritic. As a rule, pseudomembranous inflammations complicating scarlet fever, syphilis, and other infectious diseases are due to the activity of the pathogenic cocci and other bacteria, induced by the inflamed condition of the mucous membranes due to the scarlatinal or other poison. But from time to time such cases, if they have been exposed to diphtheria, may be complicated by it, and in some epidemics mixed infection is common.

Exudate Due to the Diphtheria Bacilli Contrasted with that Due to other Bacteria.—As a rule, the exudate in diphtheria is firmly incorporated with the underlying mucous membrane, and cannot be removed

without leaving a bleeding surface, at least until convalescence. The tissues surrounding the exudate are more or less inflamed and swollen. Where other bacteria produce the irritant the exudate, except in cases due to the bacillus described by Vincent, is usually loosely attached, collected in small masses, and easily removable. Exceptions, however, occur in both these diseases, so that in true diphtheria the exudate may be easily removed, and in lesions due to other bacteria the exudate may be firmly adherent.

Paralysis following a pseudomembranous inflammation is an almost positive indication that the case was one of diphtheria, although slight paralysis has followed in a very few cases in which careful cultures revealed no diphtheria bacilli. These, if not true diphtheria, must be considered very exceptional cases.

Bacteriological Diagnosis.—From the above it is apparent that fully developed characteristic cases of diphtheria are readily diagnosticated, but that many of the less marked, or at an early period undeveloped, cases are difficult to differentiate the one from the other. In these cases cultures are of the utmost value, since they enable us to isolate those in which the bacilli are found, and to give preventive infections of antitoxin to both the sick and those in contact with them, if this has not already been done. As a rule, cultures do not give us as much information as to the gravity of the case as the clinical appearances, for by the end of twenty-four to forty-eight hours the extent of the disease is usually possible of determination. The reported absence of bacilli in a culture must be given weight in proportion to the skill with which the culture was made, the suitableness of the media, the location of the disease, and the knowledge and experience of the one who examined it.

Diphtheria does not occur without the presence of the diphtheria bacilli; but there have been many cases of diphtheria in which, for one or another reason, no bacilli were found in the cultures by the examiner. In many of these cases later cultures revealed them. In a convalescent case the absence of bacilli in any one culture indicates that there are certainly not many bacilli left in the throat. Only repeated cultures can prove their total absence.

TECHNIQUE OF THE BACTERIOLOGICAL DIAGNOSIS. *Collection of the Blood Serum and its Preparation for Use in Cultures.*—A covered glass jar, which has been thoroughly cleansed with hot water, is taken to the slaughter-house and filled with freshly shed blood from a calf or sheep. The blood is received directly in the jar as it spurts from the cut in the throat of the animal. After the edge of the jar has been wiped it is covered with the lid and set aside, where it may stand quietly until the blood has thoroughly clotted. The jar is then carried to the laboratory and placed in an ice-chest. If the jar containing the blood is carried about before the latter has clotted, very imperfect separation of the serum will take place. It is well to inspect the blood in the jar after it has been standing a few hours, and, if the clot is found adhering to the sides, to separate it by a rod. The blood is allowed to remain

twenty-four hours on the ice, and then the serum which surrounds the clot is syphoned off by a rubber tube and mixed with one-third its quantity of nutrient beef-broth, to which 1 per cent. glucose has been added. This constitutes the Loeffler blood-serum mixture. This is poured into tubes, which should be about four inches in length and one-half of an inch in diameter, having been previously plugged with cotton and sterilized by dry heat at 150° C. for one hour. Care should be taken in filling the tubes to avoid the formation of air bubbles, as they leave a permanently uneven surface when the serum has been coagulated by heat. To prevent this the end of the pipette or funnel which contains the serum should be inserted well into the test-tube. About 3 c.c. are sufficient for each tube if the small size is employed, if not 5 c.c. are required. The tubes, having been filled to the required height, are now to be coagulated and sterilized. They are placed slanted at the proper angle and then kept for two hours at a temperature just below 95° C. For this purpose a Koch serum coagulator or a double boiler serves best, though a steam sterilizer will suffice. If the latter is used a wire frame must be arranged to hold the tubes at the proper inclination, and the degree of heat must be carefully watched, as otherwise the temperature may go too high, and if the serum is actually boiled the culture medium will be spoiled. After sterilization by this process the tubes containing the sterile, solidified blood serum can be placed in covered tin boxes, or stopped with sterile paraffined corks and kept for months. The serum thus prepared is quite opaque and firm.

Swab for Inoculating Culture Tubes.—The swab we use to inoculate the serum is made as follows: A stiff, thin, iron rod, six inches in length, is roughened at one end by a few blows of a hammer, and about this end a little absorbent cotton is firmly wound. Each swab is then placed in a separate glass tube, and the mouths of the tubes are plugged with cotton. The tubes and rods are then sterilized by dry heat at about 150° C. for one hour, and stored for future use. These cotton swabs have proved much more serviceable for making inoculations than platinum-wire needles or wooden sticks, especially in young children and in laryngeal cases. It is easier to use the cotton swab in such cases, and it gathers up so much more material for the inoculation that it has seemed more reliable.

For convenience and safety in transportation "culture outfits" have been devised, which consist usually of a small wooden box containing a tube of blood serum, a tube holding a swab, and a record blank. These "culture outfits" may be carried or sent by messenger or express to any place desired.

Directions for Inoculating Culture Tubes with the Exudate.—The patient is placed in a good light, and, if a child, properly held. The swab is removed from its tube, and, while the tongue is depressed with a spoon, is passed into the pharynx (if possible, without touching the tongue or other parts of the mouth) and is rubbed gently but firmly against any visible membrane on the tonsils or in the pharynx, and

then, without being laid down, the swab is immediately inserted in the blood-serum tube, and the portion which has previously been in contact with the exudate is rubbed a number of times back and forth over the whole surface of the serum. This should be done thoroughly, but it is to be gently done, so as not to break the surface of the serum. The swab should then be placed in its tube, and both tubes, thin cotton plugs having been inserted, are reserved for examination or sent to the laboratory or collecting station (as in New York City). If sent to the health department laboratories for examination the blank forms of report which usually accompany each "outfit" should be filled out and forwarded with the tubes.

Where there is no visible membrane (it may be present in the nose or larynx) the swab should be thoroughly rubbed over the mucous membrane of the pharynx and tonsils, and in the nasal cavities, and a culture made from these. In very young children care should be taken not to use the swab when the throat contains food or vomited matter, as then the bacteriological examination is rendered more difficult. Under no conditions should any attempt be made to collect the material shortly after the application of strong disinfectants (especially solutions of corrosive sublimate) to the throat.

Examination of Cultures.—The culture tubes which have been inoculated, as described above, are kept in an incubator at 37° C. for twelve hours, and are then ready for examination. When great haste is required, even five hours will often suffice for a sufficient growth of bacteria for a skilled examiner to decide as to the presence or absence of the bacilli. On inspection it will be seen that the surface of the blood serum is dotted with numerous colonies, which are just visible. No diagnosis can be made from simple inspection; if, however, the serum is found to be liquefied or shows other evidences of contamination the examination will probably be unsatisfactory.

In order to make a microscopic preparation a clean platinum needle is inserted in the tube and quite a large number of colonies are swept with it from the surface of the culture medium, a part being selected where small colonies only are found. A sufficient amount of the bacteria adherent to the needle is washed off in the drop of water previously placed on the cover-glass and smeared over its surface. The bacteria on the glass are then allowed to dry in the air. The cover-glass is then passed quickly through the flame of a Bunsen burner or alcohol lamp, three times in the usual way, covered with a few drops of Loeffler's solution of alkaline methylene blue, and left without heating for five to ten minutes. It is then rinsed off in clear water, dried, and mounted in balsam. When other methods of staining are desired they are carried out in the proper way.

In the great majority of cases one of two pictures will be seen with the $\frac{1}{12}$ oil-immersion lens—either an enormous number of characteristic Loeffler bacilli, with a moderate number of cocci, or a pure culture of cocci, mostly in pairs or short chains. (See Streptococcus.) In a few cases there will be an approximately even mixture of Loeffler bacilli

and cocci, and in others a great excess of cocci. Besides these, there will be occasionally met preparations in which, with the cocci, there are mingled bacilli more or less resembling the Loeffler bacilli. These bacilli, which are usually of the pseudodiphtheria type of bacilli (see Fig. 63), are especially frequent in cultures from the nose.

In not more than one case in twenty will there be any serious difficulty in making the diagnosis, if the serum in the tube was moist and had been properly inoculated. In such a case another culture must be made or the bacilli plated out and tested in pure culture.

Direct Microscopic Examination of the Exudate.—An immediate diagnosis without the use of cultures is often possible from a microscopic examination of the exudate. This is made by smearing a slide or cover-glass with a little of the exudate from the swab, drying, heating, staining, and examining it microscopically. This examination, however, is much more difficult, and the results are more uncertain than when the covers are prepared from cultures. The bacilli from the membrane are usually less typical in appearance than those found in cultures, and they are mixed with fibrin, pus, and epithelial cells. They may also be very few in number in the parts reached by the swab, or bacilli may be met with which closely resemble the Loeffler bacilli in appearance, but which differ greatly in growth and in other characteristics, and have absolutely no connection with them. When in a smear containing mostly cocci a few of these doubtful bacilli are present, it is impossible either to exclude or to make the diagnosis of diphtheria with certainty. Although in some cases this immediate examination may be of the greatest value, it is not a method suitable for general use, and should always be controlled by cultures.

When carried out in the best manner an experienced bacteriologist may obtain remarkably accurate results. Higley in New York in a series of consecutive throat cases made the same diagnosis from the direct examination of smears as the Health Department laboratory made from the culture. To get the exudate he used a probe armed with a loop of heavy copper wire which has been so flattened as to act as a blunt curette. He makes thus thin smears from the exudate. After drying and fixing by heat the smears are stained for five seconds in a solution made by adding five drops of Kühnes carbolic methylene blue in 7 c.c. of tap-water. After washing and drying stain for one minute in a solution of 10 drops of carbol-fuchsin in 7 c.c. of water. The dilute solution should be freely prepared. The diphtheria bacilli will appear as dark-red or violet rods, and their contour, mode of division, and arrangement are manifest.

Animal Inoculation as a Test of Virulence.—If the determination of the virulence of the bacilli found is of importance, animal inoculations must be made. Experiments on animals form the only method of determining with certainty the virulence of the diphtheria bacillus. For this purpose, alkaline broth cultures of forty-eight hours' growth should be used for the subcutaneous inoculation of guinea-pigs. The amount injected should not be more than one-fifth per cent. of the body-

weight of the animal inoculated, unless controls with antitoxin are made. In the large majority of cases, when the bacilli are virulent, this amount causes death within seventy-two hours. At the autopsy the characteristic lesions already described are found. Bacilli which in cultures and in animal experiments have shown themselves to be characteristic may be regarded for practical purposes as certainly true diphtheria bacilli, and as capable of producing diphtheria in man under favorable conditions.

For an absolute test of specific virulence antitoxin must be used. A guinea-pig is injected with antitoxin, and then this and a control animal, with 2 c.c. of a broth culture of the bacilli to be tested, if the guinea-pig which received the antitoxin lives, while the control dies, it was surely a diphtheria bacillus which killed by means of diphtheria toxin—or, in other words, not simply a virulent bacillus, but a virulent diphtheria bacillus. When the bacilli to be tested grow poorly in a simple nutrient bouillon they should be grown in bouillon to which one-third its quantity of ascitic fluid has been added. Quite a number of bacilli have been met with which killed 250-grm. guinea-pigs in doses of 2 to 15 c.c., and yet were unaffected by antitoxin. These bacilli, though slightly virulent to guinea-pigs, produce no diphtheria toxin, and so cannot, to the best of our belief, produce diphtheria in man.

CHAPTER XVIII.

THE BACILLUS AND THE BACTERIOLOGY OF TETANUS.

TETANUS is a disease which is characterized by a gradual onset of general spasm of the voluntary muscles, commencing in man most often in those of the jaw and neck, and extending in severe cases to all the muscles of the body. The disease is usually associated with a wound received from four to fourteen days previously.

In 1884 Nicolaier, under Flügge's direction, produced tetanus in mice and rabbits by the subcutaneous inoculation of particles of garden earth. The Italians, Carle and Rattone, had just before demonstrated that the pus of an infected wound from a person attacked with tetanus could produce the same disease in rabbits, and showed that the disease was transmissible by inoculation from these animals to others. Finally, Kitasato, in 1889, obtained the bacillus of tetanus in pure culture and described his method of obtaining it and its biological characters.

Occurrence in Soil, etc.—The tetanus bacillus occurs in nature as a common inhabitant of the soil, at least in places where manure has been thrown, being abundant in many localities, not only in the superficial layers, but also at the depth of several feet. It has been found in many different substances and places—in hay-dust, in horse and cow manure, in the mortar of old masonry, in the dust from horses' hair; in the dust in rooms of houses, barracks, and hospitals; in the air, and in the arrow poison of certain savages in the New Hebrides, who obtained it by smearing the arrow-heads with dirt from crab holes in the swamps.

Morphology.—In young gelatin cultures the bacilli appear as motile, slender rods, with rounded ends, 0.5μ to 0.8μ in diameter by 2μ to 4μ in length, usually occurring singly, but, especially in old cultures, often growing in long threads. They form round spores, thicker than the cell (from 1μ to 1.5μ in diameter), occupying one of its extremities and giving to the rods the appearance of small pins (Fig. 84).

Staining.—It is *stained* with the ordinary aniline dyes, and is not decolorized by Gram's method. The spores are readily stained and may be demonstrated by double-staining with Ziehl's method. The flagella are fairly easily stained in very young cultures.

Biology.—An *anaërobic, liquefying, moderately motile* bacillus. It has abundant flagella. *Forms spores*, and in the spore stage it is not motile. It grows slowly at temperatures from 20° to 24° C., and best at 37° C., when within twenty-four hours it forms spores. It will not in pure culture grow in the presence of oxygen or carbon dioxide gas, but grows well in an atmosphere of hydrogen gas. With certain other bacteria the tetanus bacillus grows luxuriantly in the presence of oxygen.

Growth in Media.—The bacillus of tetanus grows in ordinary nutrient gelatin and agar of a slightly alkaline reaction. The addition to the media of 1.5 per cent. of glucose causes the development to be more rapid and abundant. It also grows abundantly in alkaline bouillon under an atmosphere of hydrogen. On *gelatin plates* the colonies develop slowly; they resemble somewhat the colonies of the *bacillus subtilis*, and have a dense, opaque centre surrounded by fine, diverging rays. Liquefaction takes place more slowly, however, than with the bacillus subtilis, and the resemblance to these colonies is soon lost.

The colonies on *agar* are quite characteristic. To the naked eye they present the appearance of light, fleecy clouds; under the microscope, a tangle of fine threads.

The *stab cultures in gelatin* exhibit the appearance of a cloudy, linear mass, with prolongations radiating into the gelatin from all sides. Liquefaction takes place slowly, generally with the production of gas. In *stab cultures in agar* a growth occurs not unlike in structure that of

FIG. 84

Tetanus bacilli with spores in distended ends. × 1100 diameters.

a miniature pine-tree. *Alkaline bouillon* is rendered somewhat turbid by the growth of the tetanus bacillus. In all cases a production of gas results, accompanied by a characteristic and very disagreeable odor. It develops in *milk* without coagulating it.

Resistance of Spores to Deleterious Influences.—The spores of the tetanus bacillus are very resistant to outside influences; in a desiccated condition they retain their vitality for years, and are not destroyed in two and a half months when present in putrefying material. They withstand an exposure of one hour to 80° C., but are killed by an exposure of five minutes at 100° C. to live steam. They resist the action of 5 per cent. carbolic acid for ten hours. A 5 per cent. solution of carbolic acid, however, to which 0.5 per cent. of hydrochloric acid has been added, destroys them in two hours. They are killed when acted upon for three hours by bichloride of mercury (1:1000), and in thirty minutes when 0.5 per cent. HCl is added to the solution. Silver nitrate solutions destroy the spores in one minute in 1 per cent. solution and in about five minutes in 1:1000 solution.

Isolation of Pure Cultures.—The growth of the tetanus bacillus in the animal body is comparatively scanty, and is usually associated with that of other bacteria; hence, the organism is difficult to obtain in pure culture. The method of procedure proposed by Kitasato, which, however, is not always successful, consists in inoculating slightly alkaline nutrient agar or glucose bouillon with the tetanus-bearing material (pus or tissue from the inoculation wound), keeping the culture under anaërobic conditions for twenty-four to forty-eight hours at a temperature of 37° C., and, after the tetanus spores have formed, heating it for one-half an hour at 80° C., to destroy the associated bacteria. The spores of the tetanus bacillus are able to survive this exposure, so that when anaërobic cultures are then made in the usual way the tetanus colonies develop. When the tetanus bacilli are the only spore-bearing bacteria present, pure cultures are readily obtained; when other spore-bearing anaërobic bacteria are present, the isolation of a pure culture may be a matter of difficulty.

Pathogenesis.—In mice, guinea-pigs, rabbits, horses, goats, and a number of other animals inoculations of pure cultures of the tetanus bacillus cause typical tetanus after an incubation of from one to three days. A mere trace of an old culture—only as much as remains clinging to a platinum needle—is often sufficient to kill very susceptible animals like mice and guinea-pigs. Other animals require a larger amount. Rats and birds are but little susceptible, and fowls scarcely at all. Man is more susceptible than any of the animals so far tested. A horse is about six times as sensitive as a guinea-pig and three hundred thousand times as sensitive as a hen. It is a remarkable fact that an amount of toxin sufficient to kill a hen would suffice to kill 500 horses. It is estimated that if 1 gram of horse requires 1 part of toxin to kill, then 1 gram of guinea-pig requires 6 parts, 1 of mouse 12, of goat 24, of dog 500, of rabbit 1500, of cat 6000, of hen 360,000. Cultures from different cases vary greatly in their toxicity. On the inoculation of less than a fatal dose in test animals a local tetanus may be produced, which lasts for days and weeks and then ends in recovery. On killing the animal there is found at autopsy, just at the point of inoculation, a hemorrhagic spot, and no changes other than these here or in the interior organs. A few tetanus bacilli may be detected locally with great difficulty, often none at all; possibly a few may be found in the region of the lymphatic glands. From this scanty occurrence of bacilli the conclusion has been reached that the bacilli of tetanus, when inoculated in pure culture, do not multiply to any great extent in the living body, but only produce lesions through the absorption of the poison which they develop at the point of infection. It has been found that pure cultures of tetanus, after the germs have sporulated and the toxins been destroyed by heat, can be injected into animals without producing tetanus. But if a culture of non-pathogenic organisms is injected simultaneously with the spores, or if there is an effusion of blood at the point of injection, or if there was a previous bruising of the tissues, the animals surely die of tetanus. Even irritating foreign

bodies have been introduced along with the spores deprived of their toxins, and tetanus did not develop; but if the wounds containing the foreign bodies became infected with other bacteria, tetanus developed and the animal died. From such experiments it seems that a mixed infection aids greatly in the development of tetanus when the infection is produced by spores.

Natural Infection.—Here the infection may be considered as probably produced by the bacilli in their spore state, and the conditions favoring infection are almost always present. A wound of some kind has occurred, penetrating at least through the skin, though perhaps of a most trivial character, such as might be caused by a dirty splinter of wood, and the bacilli or their spores are thus introduced from the soil in which they are so widely distributed. If in any given case, the tissues being healthy, the ordinary saprophytic germs are killed by proper disinfection at once, a mixed infection does not take place, and tetanus will not develop. If, however, the tissues infected be badly bruised or lacerated, the spores may develop and produce the disease. Gelatin is found to contain occasionally tetanus spores.

Occurrence and Duration of Life of Tetanus Bacilli.—With regard to the persistence of tetanus spores upon objects where they have found a resting place, Henrijean reports that by means of a splinter of wood which had once caused tetanus he was able after eleven years to again cause the disease by inoculating an animal with the infective material. The bacilli of tetanus are apparently more numerous in certain localities than in others—for example, some parts of Long Island and New Jersey, have become notorious for the number of cases of tetanus caused by small wounds—but they are very generally distributed, as the experiments on animals inoculated with garden earth have shown, and are fairly common in New York City. In some islands and countries in the tropics puerperal tetanus and tetanus in the newborn is very frequent. Tetanus bacilli are found in intestines of about 10 per cent. of horses and calves living in the vicinity of New York City.

Tetanus in Man.—Man and almost all domestic animals are subject to tetanus. On examination of an infected individual very little local evidence of the disease can be discovered. Generally at the point of infection, if there is an external wound, some pus is to be seen, in which, along with numerous other bacteria, tetanus bacilli or their spores may be found. Although rather deep wounds are usually the seat of infection, at times such superficial wounds, as an acne pustule or a vaccination, may give the occasion for infection. Not only undoubted traumatic tetanus, but also all the other forms of tetanus, are now conceded to be produced by the tetanus bacillus—puerperal tetanus, tetanus neonatorum, and idiopathic tetanus. In tetanus neonatorum infection is introduced through the navel, in puerperal tetanus through the inner surface of the uterus. It should be borne in mind that when there is no external and visible wound there may be an internal one.

Toxins of the Tetanus Bacillus.—It is evident from the localization of the tetanus bacilli at the point of inoculation and their slight mul-

tiplication at this point that they exert their action through the production of powerful toxins. These toxins are named, according to their action, the tetanospasmin and the tetanolysin. One one-hundredth of a milligram of the filtrate of an eight-day glucose bouillon culture of a fully virulent bacillus is sufficient to kill a mouse. From this filtrate, however, the active toxic substance has been obtained in a much more concentrated form. The purified and dried tetanus toxin prepared by Brieger and Cohn was surely fatal to a 15-gram mouse in a dose of 0.00000005 gram. Reckoning according to the body-weight of 75 kilograms, or 175 pounds, it would require but 0.00023 gram, or 0.23 milligram, of this toxin to prove fatal to a man. The appalling strength of tetanus toxin may readily be appreciated. By comparing it with snake poison and strychnine, Calmette has found that dried cobra venom would require 4.375 milligrams to kill a man of 70 kilograms. A fatal dose of strychnine is from 30 to 100 milligrams.

The quantity of the toxin produced in nutrient media varies according to the age of the culture, the composition of the culture fluid, reaction, completeness of the exclusion of oxygen, etc. The variation in strength is partly due to the extreme sensitiveness of the toxin, which deteriorates on keeping or on exposure to light, being also sensibly affected by most chemical reagents and destroyed by heating to 55° to 60° C. for any length of time. It retains its strength best when protected from heat, light, oxygen, and moisture. Under the best conditions the amount of toxin produced in cultures by the fifth day is such that 0.000005 c.c. is the fatal dose for a 15-gram mouse.

The tetanus cultures retain their ability to produce toxins unaltered when kept under suitable conditions; but when subjected to deleterious influences they may entirely lose it. The usual medium for the development of the toxin is a slightly alkaline bouillon containing 1 per cent. of peptone and 0.5 per cent. salt. The addition of more than a trace of sugar or glycerin is to be avoided, as the acid produced injures the toxin.

Action of Tetanus Toxin in the Body.—After the absorption of the poison there is a lapse of time before any effects are noticed. With an enormous amount, such as 30,000 fatal doses, this is about twelve hours; with ten fatal doses, thirty-six to forty-eight hours; with two fatal doses, two to three days. Less than a fatal dose will produce local symptoms. The parts first to be affected with tetanus are, in about one-third of the cases in man and usually in animals, the muscles lying in the vicinity of the inoculation—for instance, the hind foot of a mouse inoculated on that leg is first affected, then the tail, the other foot, the back and chest muscles on both sides, and the forelegs, until finally there is a general tetanus of the entire body. In mild cases, or when a dose too small to be fatal has been received, the tetanic spasm may remain confined to the muscles adjacent to the point of inoculation or infection. The symptoms following a fatal dose of toxin vary greatly with the method of injection. Intraperitoneal injection is followed by symptoms which can hardly be distinguished from those due to many other poisons. Injection into the brain is followed by restlessness and

epileptiform convulsions. The tetanus toxins undoubtedly combine readily with the cells of the central nervous system. They also combine with other tissue cells with less apparent effects. The symptoms in tetanus depend upon an increased reflex excitability of the motor cells of the spinal cord, the medulla, and pons.

Presence of Tetanus Toxin in the Blood of Infected Animals.—The blood usually contains the poison, as has been proved experimentally on animals. Neisser showed that the blood of a tetanic patient was capable of inducing tetanus in animals when injected subcutaneously. In St. Louis the serum of a horse dying of tetanus was given by accident in doses of 5 to 10 c.c. to a number of children, with the development of fatal tetanus. In this connection Bolton and Fisch showed by a series of experiments that much toxin might accumulate in the serum before symptoms became marked. Kitasato also found the serous exudates of the pleural and pericardial cavities as well as the blood of tetanic animals would cause tetanus when transferred to other animals. Ehrlich has shown that besides the predominant poison which gives rise to spasm (tetanospasmin) there exists a poison capable of producing solution of certain red blood corpuscles. This he calls tetanolysin. It was not found in all culture fluids and does not pass through in the first portion of the filtrate from a porcelain filter. Whether in actual disease this poison is ever in sufficient amount to cause appreciable harm is not known.

Tetanus Antitoxin.—Behring and Kitasato were the first to show the possibility of immunizing animals against tetanus infection. The entire procedure is analogous to immunization against diphtheria. The treatment of tetanus is directed against the action of the toxin and this is accomplished by the neutralization of the toxin by antitoxin in the body.

The immunizing experiments in tetanus have borne practical fruit, for it was through them that the principle of serum therapeutics first became known—the protective and curative effects of the blood serum of immunized animals. It was found that animals could be protected from tetanus infection by the previous or simultaneous injection of tetanus antitoxin, provided that such antitoxic serum was obtained from a thoroughly immunized animal. From this it was assumed that the same result could be produced in natural tetanus in man. Unfortunately, however, the conditions in the natural disease are very much less favorable, inasmuch as treatment is usually commenced not shortly after the infection has taken place, but only on the appearance of tetanic symptoms, when the poison has already attacked the cells of the central nervous system.

The tetanus antitoxin is developed in the same manner as the diphtheria antitoxin—by inoculating the tetanus toxin in increasing doses into horses. The toxin is produced in bouillon cultures grown anaërobically. After six to fifteen days the culture fluid is filtered through porcelain, and the germ-free filtrate is used for the inoculations. The horses receive ½ c.c. as the initial dose of a toxin of which 1 c.c. kills 250,000 grams of guinea-pig, and along with this a sufficient amount

of antitoxin to neutralize it. In five days this dose is doubled, and then every five to seven days larger amounts are given. After the third injection the antitoxin is omitted. The dose is increased as rapidly as the horses can stand it, until they support 700 to 800 c.c. or more at a time. This amount should not be injected in a single place, or severe local and perhaps fatal local tetanus may develop. After some months of this treatment the blood of the horse contains the antitoxin in sufficient amount for therapeutic use. When the animals' temperatures are normal and they have recovered from the dose of toxin last given, they are bled into sterile flasks and the serum collected. A quicker and safer method of immunization is to mix antitoxin with larger doses of toxin in the first four injections.

Technique of Testing Antitoxin Serum for Value in Antitoxin.—Tetanus antitoxin is tested exactly in the same manner as diphtheria antitoxin, except that the unit adopted by different dispensers is different. This confuses the physician and almost compels him to give a certain number of cubic centimetres without regard to the units contained. The amount of antitoxic serum which neutralizes an amount of test toxin which would destroy 40,000,000 grams of mouse contains 1 unit of antitoxin by the German standard. One large American producer considers 1 unit of antitoxin to be the amount which neutralizes only 100 grams of mouse. In the French method the amount of antitoxin which is required to protect a mouse from a dose of toxin sufficient to kill in four days is determined, and the strength of the antitoxin is stated by determining the amount of serum required to protect 1 gram of animal. If 0.001 c.c. protected a 10-gram mouse the strength of that serum would be 1:10,000. Guinea-pigs are sometimes used in place of mice. The United States government should make an official tetanus antitoxin unit in the same way it has an official diphtheria antitoxin unit. The toxin used for testing is preserved by precipitating it with saturated ammonium sulphate and drying and preserving the precipitate in sealed tubes. As required, it is dissolved in 10 per cent. salt solution as above stated. For small testing stations the best way is to obtain some freshly standardized antitoxin and compare serums with this.

Persistence of Antitoxin in the Blood.—Ransom has recently shown that the tetanus antitoxin, whether directly injected or whether produced in the body, is eliminated equally rapidly from the blood of an animal provided that the serum was from an animal of the same species. If from a different species it is much more quickly eliminated. From this we see a possible explanation of the fact that immunity in man, due to an injection of the antitoxic serum of the horse, is less persistent than immunity conferred by an attack of the disease.

The same author found some interesting facts in testing the antitoxic values of the serum of an immunized mare, of its foal, and of the milk. The foal's serum was one-third the strength of the mare's, and one hundred and fifty times that of the mare's milk. In two months the mare's serum lost two-thirds in antitoxic strength, the foal's five-sixths,

and the milk one-half. Injections of toxin were then given the mare, so that it doubled its original strength in one month. The milk increased eightfold, but the foal's continued to lose in antitoxin, although it was feeding on the antitoxic milk.

Toxin and Antitoxin in the Living Organism. Animal Experiments.—Very recently the studies of H. Meyer and Ransom as well as those of Marie and Morax in Roux's laboratory have thrown considerable light on the much disputed phenomena observed in poisoning by tetanus toxin. The investigations of Gumprecht in 1895 had made it highly probable that all the pathological symptoms were due to a poisoning of the central nervous system. The paths, however, by which the poison reached its central points of attack were still doubtful. The experiments of Meyer and Ransom and of Marie and Morax have practically proved that the poison is transported to the central nervous system by way of the motor nerves—and by no other channel. So far as the experiments are concerned, I must refer the reader to the original. These show that the essential element for the absorption and transportation of the toxin is not the nerve sheath or the lymph channels, but the axis-cylinder, the intramuscular endings of which the toxin penetrates. The poison is taken up quite rapidly. Marie and Morax were able to demonstrate the poison in the corresponding nerve trunk (sciatic) one and a half hours after the injection. Absorption, however, and conduction are dependent to a large extent on the nerves being intact. A nerve cut across takes very much longer to take up the poison (about twenty-four hours), and a degenerated nerve takes up no poison whatever. In other words, we see that section of the nerve prevents the absorption of the poison by way of the nerve channels. Similarly section of the spinal cord prevents the poison from ascending to the brain.

According to Meyer and Ransom the reason why the sensory nerves do not play any role in the conduction of the poison lies in the presence of the spinal ganglion, which places a bar to the advance of the poison. Injections of toxin into the posterior root leads to a tetanus dolorosus, which is characterized by strictly localized sensitiveness to pain.

Ascending centripetally along the motor paths the poison reaches the motor spinal ganglia on the side of inoculation; then it affects the ganglia of the opposite side, making them hypersensitive. The visible result of this is the highly increased muscle tonus—*i. e.*, rigidity. If the supply continues, the toxin next affects the nearest sensory apparatus; there is an increase in the reflexes, but only when the affected portion is irritated. In the further course of the poisoning the toxin as it ascends continues to affect more and more motor centres, and also the neighboring sensory apparatus. This leads to spasm of all the striated muscles and general reflex tetanus.

If the toxin gets into the blood the only path of absorption to the central nervous system is still by way of the motor-nerve tracts. There seems to be no other direct path, as, for example, by means of the bloodvessels supplying the central nervous system. Even after intro-

ducing the poison into the subarachnoid space, owing to the passage of the poison into the blood, there is a general poisoning and not a cerebral tetanus. This at least is the case if care has been taken during the operation to avoid injuring the brain mechanically.

If now we follow the course of the *antitoxin* in the organism as we have just done with the toxin, we find that: whereas the toxin passes directly to the nerve paths, the antitoxin when injected subcutaneously is entirely taken up by the blood-vessels by means of the lymph-vessels. From the blood it then reaches the tissues, with the fluids of which it mixes.

The complete absorption of a given quantity of antitoxin administered subcutaneously takes place rather slowly. In his animal experiments Knorr found the maximum quantity in the blood only after twenty-four to forty hours. From that time on the amount again steadily decreased, so that by the sixth day only one-third the optimum quantity was present. By the twelfth day only one-fiftieth and at the end of three weeks no antitoxin whatever could be demonstrated.

Naturally the time during which these changes take place varies with the application, the conditions of absorption, and the concentration and amount of the preparation injected. When injected intravenously the antitoxin very quickly passes into the lymph. Ransom was able to demonstrate it in the thoracic duct of a dog a few minutes after intravenous injection. *Neither the central nervous system nor the peripheral nervous tissue take up any antitoxin from the blood.* Only after very massive intravenous doses are small traces found in the cerebrospinal fluid. From this it is at once clear that passively and actively immunized animals become tetanic if the poison is injected directly into the central nervous system or into a peripheral nerve. Antitoxin injected subdurally also passes almost entirely over into the blood.

A rapid and plentiful appearance of antitoxin in the blood is dependent on the content of serum in antitoxin units. The more units, the more rapidly will the blood develop a high content of antitoxin; and the higher this is the more thoroughly will the tissues be saturated with the antitoxin.

From the foregoing it is not difficult to formulate the conditions under which an antitoxin introduced into the organism can exert its neutralizing power on the toxin. We see that the poison deposited at any given place takes either of two paths to the central nervous system, one a direct path by way of the local peripheral nerves and the other an indirect path through the lymph channels and blood to the end plates of all other motor nerves. From intact bloodvessels the antitoxin penetrates neither the substance of the peripheral nerves nor the substance of the central nervous system. Hence, only that portion can be neutralized which (*a*) still lies unabsorbed at the site of inoculation, or (*b*) which, though it has passed into the blood, has not yet been taken up by the motor-nerve endings. A curative effect can therefore result from antitoxin introduced subcutaneously or intravenously only

so long as a fatal dose of poison has not been taken up by the nerves. After this has occurred an action of the antitoxin can only then be looked for when it is injected directly into the nerve substance.

So long as the toxin circulates in the blood it is neutralized by antitoxin in about the same proportion as in test-tube experiments. By means of intravenous injections of antitoxin Ransom was able to render the blood free from toxin in a very few minutes. According to Marie and Morax toxin injected into the muscles is already demonstrable in the nerve tissue at the end of one and a half hours—*i. e.*, it has already entered the channel, where it is no longer reached by the antitoxin. There must, however, be a condition or locality in which the toxin can still be neutralized by means of large doses, though with difficulty. This is indicated among other experiments by some older researches of Dönitz. This observer injected various rabbits intravenously each with 1 c.c. of a toxin solution containing twelve fatal doses. Thereupon he determined the dose of antitoxin which, when intravenously given, would neutralize this poison after various intervals of time. The antitoxin was of such a strength that in test-tube experiments 1 c.c. of a 1:2000 solution just neutralized the amount of toxin employed. He found that at the end of two minutes double the dose required *in vitro* would still neutralize the poison; at the end of four minutes about four times the dose was required, and at the end of eight minutes ten times. When one hour had been allowed to elapse forty times the original dose just sufficed to protect the animal from death, but not from sickness. In order to explain these results, the correctness of which has been confirmed by many analogous observations, the conception "loose union of toxin" has been introduced. By this is meant a state of union between toxin and susceptible cell constituent which can still be disrupted by means of large doses of antitoxin. In this particular instance we do not need to make use of this conception, for the reason that the tetanus toxin is not at all combined during the first hour, being engaged at this time in traversing the peripheral nerve paths. Personally, I should regard it as more probable that the interval during which the toxin can still be neutralized, though with difficulty, corresponds to that time during the passage of the toxin in which after leaving the capillaries the poison is held up in the fine interstices of the connective tissue which it must penetrate before it can be taken up by the nerves.

Results of the Antitoxin Treatment in Tetanus.—Tetanus is a comparatively rare disease both in man and animals, though in some localities it is more common than in others. In New York City there are usually fifteen to thirty cases following every Fourth of July. Most of them are caused by infection through blank-cartridge wounds. Recovery sometimes follows from the ordinary symptomatic treatment or without treatment at all, so that the statistics of cures of the disease by the injection of antitoxic serum must be very carefully sifted before they can be accepted as reliable. Lambert, who a few years ago made an exhaustive study of tetanus, states that in a total of 114 cases

of this disease treated with antitoxin, according to published and unpublished reports, there was a mortality of 40.35 per cent. Of these, 47 were acute cases—that is, cases with an incubation period of eight days or less and with rapid onset, or cases with a longer period of incubation, but intensely rapid onset of symptoms; of these the mortality was 74.46 per cent. Of the chronic type—those with an incubation period of nine days or more, or those with shorter incubation with slow onset—there were 61 cases, with a mortality of 16.39 per cent. With a still larger number of cases the results indicate that with tetanus antitoxin about 20 per cent. better results are obtained than without.

Treatment of Tetanus by Antitoxin.—For immunization, 10 c.c. of a serum of medium strength will suffice unless the danger seems great, when the injection is repeated at the end of a week. For treatment, it is well to begin with 30 to 50 c.c., and then, according to the severity of the case, give from 20 to 50 c.c. each day until the symptoms abate. In the gravest cases no curative effect will be noticed from the serum. The first injections should be made intravenously, or partly intravenously and partly into the spinal canal through lumbar puncture. Later, injections should be made subcutaneously or intravenously. Besides these, injections should be made into all the nerve trunks leading from the infected region. These injections should be made as near the trunk as possible and distend the nerve so as to partly neutralize and partly mechanically interrupt the passage of toxin to the cord or brain. In New York City Rogers has had good results by following these cases. He has also injected the antitoxin directly into the spinal cord. The method of injecting from 3 to 15 c.c. of antitoxic serum into the lateral ventricles has not, in the writer's opinion, shown itself to be equal to the intravenous and intraspinal and intraneural methods. No bad results have followed the injections when the serum was sterile and the operation was performed aseptically; but several brain abscesses have already followed the intracerebral injections.

The striking results which have been obtained, particularly in veterinary practice, with the prophylactic injection of tetanus antitoxin, would seem to warrant the treating of patients with immunizing doses of serum—at least, in neighborhoods where tetanus is not uncommon—when the lacerated and dirty condition of their wounds may indicate the possibility of a tetanus infection.

Differential Diagnosis.—The differential diagnosis of the bacillus of tetanus is, generally speaking, not difficult, inasmuch as animal inoculation affords a sure test of the specific organism. No other microorganism known produces similar effects to the tetanus bacillus, nor is any other neutralized by tetanus antitoxin. The other characteristics also of this bacillus are usually distinctive, though microscopic examination alone cannot be depended on to make a differential diagnosis. Difficulty arises when other anaërobic or aërobic bacilli, almost morphologically identical with the tetanus bacillus, are encountered which are non-pathogenic, such as the *bacillus pseudotetanicus anaërobius*, already mentioned, and the *bacillus pseudotetanicus aërobius*. It

it possible, however, that both these bacilli, when characteristic in cultures, are only varieties of the tetanus bacillus, which, under unfavorable conditions of growth, have lost their virulence. These non-virulent types do not, as a rule, have spores absolutely at their ends, and the spores themselves are usually more ovoid than those in the true tetanus bacilli.

Methods of Examination in a Case of Tetanus. (*a*) *Microscopic.*—From every wound or point of suppuration film preparations should be made and stained with the usual dyes. The typical spore-bearing forms are looked for, but are usually not found. At the same time other bacteria are noted if present.

(*b*) *Cultures.*—Bits of tissue, pus, cartridge wads, etc., are collected and dropped into glucose bouillon contained in small flasks or tubes. This bouillon should be slightly alkaline, be free from oxygen, and protected from oxygen. A simple way is to cover the bouillon with liquid or semisolid paraffin, and thus boil it. Cultures placed in such protected bouillon grow readily.

(*c*) *Inoculation.*—Material from the wound is inoculated into mice or guinea-pigs subcutaneously.

CHAPTER XIX.

THE COLON BACILLUS GROUP, PARACOLON, PARATYPHOID DYSENTERY AND PARADYSENTERY BACILLI.

The Colon Bacillus Group.

THE first organism of this group was described by Emmerich (1885), who obtained it from the blood, various organs, and intestinal discharges of cholera patients at Naples. Similar organisms were afterward found by Escherich (1886) in the feces of healthy, milk-fed infants. It has since been demonstrated that members of the colon group are normal inhabitants of the intestines of man and of most of the lower animals.

Morphology.—The bacillus coli varies considerably in its morphology, according to the sources and the culture media from which it is obtained. The typical form (Fig. 85) is that of short rods with rounded ends, from 0.4μ to 0.7μ in diameter by 1μ to 3μ in length; sometimes, especially where the culture media are not suitable for their growth, the rods are so short as to be almost spherical, resembling micrococci in appearance, and, again, they are somewhat oval in form or are seen as threads of 6μ or more in length. The various forms may often be associated in the same culture. The bacilli may occur as single cells or as pairs joined end-to-end, rarely as short chains. Capsules, though present, are not shown by the ordinary methods.

FLAGELLA.—Upon some varieties seven or eight flagella have been demonstrated, upon others none. The flagella are shorter and more delicate than those characteristic of the typhoid bacilli. There is nothing in the morphology of this bacillus sufficiently characteristic for its identification, for in this respect it simulates many other organisms.

Staining.—The colon bacillus *stains* readily with the ordinary aniline colors; it is always decolorized by Gram's method.

Biology.—It is an *aërobic, facultative anaërobic, non-liquefying* bacillus. It develops best at 37° C., but grows well at 20° C. and slowly at 10° C. It is usually motile, but the movements in some of the cultures are so sluggish that a positive opinion is often difficult. In fresh cultures frequently, only one or two individuals out of many show motility.

Cultivation.—The colon bacillus develops well on all the usual culture media. Its growth on them is usually more abundant than that of the typhoid bacillus or the dysentery bacillus, but the difference is not sufficient for a differential diagnosis. Although it grows best aërobically, yet it grows well anaërobically, especially in media containing fermentable sugars.

Gelatin.—In gelatin plates colonies are developed in from eighteen to thirty-six hours. They resemble greatly the colonies of the typhoid bacillus, except that many of them are somewhat larger and more opaque. When located in the depths of the gelatin and examined by a low-power lens they are at first seen to be finely granular, almost homogeneous, and of a pale yellowish to brownish color; later they become larger, denser, darker, and more coarsely granular. In shape they may be round, oval, or whetstone-like. The superficial colonies appear as small, dry, irregular, flat, blue-white points, that are commonly somewhat dentated at the margins. When the gelatin is not firm the margins of many colonies are broken by outgrowths, which are rather characteristic of colon bacilli. Under the same conditions the colonies of typhoid bacilli usually show thread-like outgrowths.

Fig 85

Colon bacilli. Twenty-four-hour agar culture. $\times$ 1100 diameters.

In stab cultures on gelatin the growth usually takes the form of a nail with a flattened head, the surface extension generally reaching out rapidly to the sides of the tube.

Nutrient Agar.—In plate cultures: Surface colonies mostly circular, finely granular, and rather opaque. The deep colonies are apt to have protuberances. There are no marked differences between colonies of colon and typhoid bacilli. In streak cultures an abundant, soft, white layer is quickly developed, but the growth is not characteristic.

Bouillon.—In bouillon the bacillus coli produces diffuse clouding with sedimentation; in some cultures a tendency to pellicle formation on the surface is occasionally seen.

Potato.—On potato the growth is rapid and abundant, appearing after twenty-four to thirty-six hours in the incubator as a yellowish-brown to dark cream-colored deposit covering the greater part of the surface. But there are considerable variations from the typical growth on potato; there may be no visible growth at all, or it may be scanty and of a white color. These variations are due at times to the bacillus, but more often to variations in the potato.

COAGULATED SERUM.—The serum is not liquefied. On this medium grayish, uncharacteristic colonies are developed.

MILK coagulates in from four to ten days at 20° C., and in from one to four days at 37° C. Coagulation is aided by the addition of peptone; it is prevented by constant addition of alkalies. The acids formed are lactic, acetic, formic, and succinic acids. Coagulation is due principally but not altogether to acids. A ferment is produced which is capable of causing coagulation in the presence of lime salts, especially in acid solutions. It is evident also that the nature of this coagulation is more closely related to coagulation fermentation than to simple acid fermentation, from the fact that colon coagulation forms a compact mass which is difficultly soluble in alkalies, and contains much insoluble residue; and further, that in serum coagulated by colon bacilli a proteid body corresponding to fermentation albumin is found.

In addition to albumose it has been shown that milk serum, after colon coagulation, contains a substance possessing the reaction of peptone, which is not contained in the original milk. Similar albumin cleavage products are also formed in cultures of bacillus coli in sugar-free ascitic fluid; it cannot be assumed, therefore, that colon acidification of milk, as such, produces this proteolysis. The colon bacilli also differ from typhoid bacilli in that their action on milk-sugar is more intense, and, since they can grow in greater acidity, more lasting. The difference, therefore, is quantitative rather than qualitative.

Chemical Activities. BEHAVIOR TOWARD CARBOHYDRATES.—In cultures of colon bacilli many carbohydrates, especially sugars, become fermented. Among these are:

Hexoses, $C_6H_{12}O_6$ (glucose, mannose, fructose, galactose).
Pentoses, $C_5H_{10}O_5$ (arabinose, xylose).
Binoses, $C_{12}H_{22}O_{11}$ (saccharose, lactose, maltose, melitose).

Also the higher alcohols:

Hexatomic alcohol (mannit, dulcit, sorbit).
Pentatomic alcohol (erytherit).
Triatomic alcohol (glycerin).

The varieties included under colon bacilli vary in their action on some of the sugars; some ferment cane-sugar, others do not. All ferment glucose. For fermentation they require nitrogenous substances which can be utilized as food by the bacteria, a suitable temperature (best at 30° to 35° C.), absence of deleterious substances.

The products derived from the splitting of the carbohydrates are as follows: From glucose, saccharose, arabinose, and galactose there is mainly produced *lævo*-lactic acid along with 5 to 25 per cent of *dextro*-lactic acid; mannit, on the contrary, yields only *lævo*-lactic acid. The lactic acid formed, however, corresponds to only one-half the quantity of sugar present. The by-products are: gaseous compounds, alcohol, succinic, acetic, and formic acids. The most important fermentation products, both qualitatively and quantitatively, are produced from grape-sugar, probably, according to the following reaction:

$$\underset{\text{Grape-sugar.}}{2C_6H_{12}O_6} + \underset{\text{Water.}}{H_2O} = \underset{\text{Lactic acid.}}{2C_3H_6O_3} + \underset{\text{Acetic acid.}}{CH_3COOH} + \underset{\text{Ethyl alcohol.}}{C_2H_5COOH} + \underset{\text{Carbonic acid.}}{2CO_2} + \underset{\text{Hydrogen.}}{2H_2}$$

Acid Production from Sugars by Colon Bacilli.—When sufficient sugar is present the amount of acid produced is quite uniform. In many it proceeds until the acidity is sufficient in quantity to stop the growth of the bacilli. In milk this acidity becomes about 12 per cent. $\frac{n}{10}$ acid to phenolphthalein. There are reasons to think that lactic acid is first produced and that from this other acids and products develop. Under aërobic conditions lactic is produced in excess of acetic acid, while in the absence of oxygen the reverse is apt to be true.

Gas Production.—When colon bacilli are grown in a solution of glucose, CO_2 and H_2 are produced, $1CO_2$ to $1H_2$ up to $1CO_2$ to $3H_2$. Anaërobic conditions aid gas formation. Some colon varieties produce gas from no sugars and some from a few only. Nearly all produce gas from glucose, and about 60 per cent. of varieties produce gas from milk-sugar. Very slight traces of gases other than H and CO_2 are produced. The amount of gas varies in different varieties; the closed arm of the tube half-filled, and the H and CO_2 in the proportion 2 to 1, is the characteristic type. It is also true of Gärtner's B. enteritidis. In another type the whole of the closed arm is filled,—$H_2:CO_2=1:2$ or 3. This type is usually called bacterium cloacæ. In a third type the arm is nearly filled,—$H_2:CO_2=1:1$. This type is the bacterium lacticum aërogenes.

Concerning the gas and acid produced the following rules have been established:

1. The quantity produced varies with the amount of bacteria and their activity as well as with the type of organism.
2. It depends upon the living organisms.
3. The products have a less heat value than the sugar from which they were formed.
4. The fermentation is not a simple hydrolytic action, but one in which combinations between the C and O atoms are sundered and formed. This is not an oxidation process, but a change through breaking down—that is, a true decomposition. What oxidation takes place is chiefly due to the oxygen liberated from splitting the sugar molecules.

Use of Sugar Litmus Agars to Differentiate between Colon and Typhoid Bacilli.

List of sugars.	Color of media after 24 hours' growth of culture.	
	Colon bacillus.	Typhoid bacillus.
Grape-sugar	Red.	Red.
Saccharose	Red.	Red.
Mannite	Red.	Red.
Cane-sugar	Blue.	Blue.
Maltose	Red or moderately red.	Red or pink.
Milk-sugar	Red.	Blue.
Dextrin	Blue.	Violet blue.

To bouillon used for acid and gas formation no sodium hydrate or carbonate should be added.

EFFECT OF COLON BACILLI IN NITROGENOUS COMPOUNDS.—Colon bacilli do not appreciably liquefy gelatin nor peptonize any albumins. They do, however, break down some of the higher nitrogenous compounds into smaller atom groups. The first noted of these compounds was indol, $C_6H_4 \left\langle \begin{matrix} NH \\ CH \end{matrix} \right\rangle CH$. This is one of the most important products of colon activity, although a few varieties lack it. (Witte's peptone solution is used as test.) Sugars interfere with indol production, as also does the absence of oxygen. The maximum amount of indol is present about the tenth day. The test is carried out by adding 1 c.c. of 0.02 per cent. potassium nitrite to 10 c.c. of culture fluid, and then some concentrated sulphuric acid. To prevent confusion with other colors a little amyl alcohol is added and shaken. In the intestinal canal in health very little indol appears to be produced by colon bacilli. Sulphuretted hydrogen is liberated from sugar-free fermentable proteid substances. Mercaptan and sometimes skatol have been noted in peptone solution cultures. The colon bacillus liquefies minute quantities of gelatin, but so little as to be inappreciable.

The colon bacillus can make use of simpler nitrogen compounds than the typhoid bacillus, and can grow well in media such as that of Uschinsky. In urine some colon cultures produce a slight fermentation, yielding ammonium carbonate. By some this is believed not to be due to urea fermentation. Lactose-litmus-urine agar, after twenty-four hours' acidity, often becomes alkaline.

In media containing fermentable sugars and proteid substances simultaneous action takes place on both with the production of both alkalies and acids.

Effect on Fats.—No action has been noted.

Reduction Processes. REDUCTION OF PIGMENTS.—The action on certain pigments which are reduced to colorless products and intermediate colors is more vigorous than that of typhoid bacilli. This effect occurs in litmus bouillon in the closed arm of the tube, in bouillon and agar (not in gelatin), indigo-sodium-sulphate-methyl blue, in sugar media, etc.

REDUCTION OF INORGANIC SALTS.—From nitrates coli under certain conditions produce nitrites and from them free nitrogen as follows:

$$2NaNO_3 + H_2O = 2NaOH + N_2 + O_5.$$

The development of pigments (brownish, greenish, and yellowish), odorous substances, and toxins has been noted, especially on potato. Besides ammonia and the fatty and oxy-fatty acids other bad-smelling products, but little understood, are formed at times.

Toxins.—The bodies of dead colon bacilli contain pyogenic substances, and others which, injected into the circulation, produce paralysis of the striped muscle fibres, convulsions, coma, and death. Extracts from some cultures produce irritation of the mucous membranes of the large intestines with dysenteric symptoms.

Growth with Other Bacteria.—Colon bacilli grown with typhoid bacilli are hindered in their gas production and indol formation, while the

latter soon die out. (Typhoid bacilli usually die out in the filtrate of colon culture.) Colon varieties differ as to whether they increase or not in the old culture fluids of other broth colon cultures. The colon bacilli act antagonistically to many of the proteolytic bacteria in the intestinal tract, and so inhibit alkaline putrefaction by them. In milk the same antagonism exists, probably because of the acidity caused by the colon growth.

Reaction to Temperature.—Growth takes place best at 37° C.; the range is from 5° to 45° C. Colon bacilli are killed at 60° C. in from five to fifteen minutes. Frozen in ice a large proportion die, but some resist for six months. In liquid air 95 per cent. are killed in two hours.

Resistance to Drying.—The colon bacillus is quite resistant. Simple drying destroys the majority of organisms dried at any one time, but some bacilli of the number dried may remain alive, especially when held in the texture of threads, for five to six months, or all may die in forty-eight hours.

Effect of Light.—In water exposed to direct sunlight colon bacilli are killed in from one to six hours. In diffuse light a moderate effect only is produced. They are fairly resistant to Roentgen rays, quite so to the electric current.

Effect of Acids.—The colon bacilli grow in a wider range of acids and alkalies than most other bacteria. They develop in from 0.2 to 0.4 per cent. of mineral acids in from 0.3 to 0.45 per cent. of vegetable acids, and in from 0.1 to 0.2 per cent. of alkalies.

To most antiseptics they are resistant, growing feebly even in 1:5000 formaldehyde. They grow in from 6 to 8 per cent. of salt and live in concentrated solutions.

Effect of Animal Fluids and Juices.—Gastric juice kills colon bacilli when not protected or too greatly diluted by food. They grow in bile and in the intestinal juices. Fresh rabbit and dog blood kills colon bacilli, as also but more quickly typhoid bacilli. Calf and horse serum are said to be inactive.

Virulence of Colon Bacilli. Pathogenic Properties in Animals.—The virulence varies with the culture and the time since its recovery from the intestines. Other things being equal, it is more virulent from an intestinal inflammation. From severe diarrhœa the colon bacilli in 0.25 c.c. bouillon culture may kill guinea-pigs if given intraperitoneally, while from healthy bowel 2.5 c.c. are usually required. Smaller amounts are fatal to white mice.

Increase of Virulence Outside the Body.—It has been found by several observers that in fermenting fecal matter a marked increased virulence takes place, so that infection is produced when received by man. Some investigators claim that by growing the colon bacilli with typhoid bacilli and with the pyogenic cocci increase of virulence occurs. This is improbable outside the body. The lesions following injection with colon bacilli do not differ from those produced by the typhoid bacilli.

Pathogenesis.—The lesions present in intestinal infection are those of enteritis; the duodenum and jejunum are found to contain fluid, the spleen is somewhat enlarged, and there is marked hyperæmia and ecchymosis of the small intestines, together with swelling of Peyer's patches.

Intraperitoneal and intravenous inoculation of guinea-pigs and rabbits may also produce death, which, when it follows, usually takes place within the first forty-eight hours, accompanied by a decided fall of temperature, the symptoms of enteritis, diarrhœa, etc., and finally fibropurulent peritonitis.

When subcutaneous inoculations of mice and guinea-pigs are made it requires the introduction of much larger quantities of the cultures to produce infection; in rabbits this is followed only by abscess formation at the point of inoculation. Dogs and cats are similarly affected.

Albarran and Hallé have caused cystitis and pyelonephritis by direct injections into the bladder and ureters, the urine being artificially suppressed; Chassin and Roger produced angiocholitis and abscess of the liver in the same way. Akermann produced osteomyelitis in young rabbits by intravenous injections of cultures.

From experiments on animals it would, therefore, appear that the true explanation of the pathogenesis of the colon bacillus is undoubtedly to be found in the toxic effects of the chemical substance and products of the cells.

Immunization.—Immunization against colon bacillus infection is produced in the same way and to the same degree as in typhoid bacillus infection. The serum from a horse receiving a number of different strains is required, if protection from all members of the colon group is desired. The serum is not at present employed therapeutically.

Occurrence in Man and Animals during Health.—The bacillus coli communis is a common inhabitant of the intestinal canal in man and in almost all domestic animals. It is also found at times in wild animals and appears at times to develop in fishes.

Occurrence Outside of the Intestines.—Colon bacilli are found wherever human or animal feces are carried. In water a very few colon bacilli, less than one to each 10 c.c., are not sufficient to give rise to the suspicion that it is contaminated. Even 1 per c.c. does not show human contamination, since such a number could equally well come from manure spread on fields. Colon bacilli are apt to be found in everything which comes in contact with man or animals, or dust where they have been.

Variation in Morphological and Biological Characters.—By subjecting them to higher than normal temperature, to long growth in old culture fluids, to the action of weak antiseptics, and to the passage through animals, colon bacilli have been changed so as to lose the power to make indol and ferment sugars with gas production. They have not, however, been made to approach typhoid bacilli in their agglutination characteristics or in their motility. The change has simply been to weaken the characteristics typical of colon, and this gives them appearances similar to typhoid bacilli, but in parasitic ways they are still quite different.

To the colon group the following characteristics are essential: short

rod form; relatively good growth on media; decolorization by Gram; the lack of appreciable liquefaction of gelatin; the facultative properties; indol formation; sugar fermentation; milk coagulation; motility; pathogenic properties in animals to greater or less extent. The saprophytic life of the colon bacillus leads to gradual variations according to conditions.

Association with Other Bacteria in the Intestines.—In infant stools we usually find in ordinary cultures only bacterium coli and bacterium lactis aërogenes. Both fail to ferment albumin, and both markedly ferment carbohydrates. The bacterium lactis aërogenes is most abundant in the upper intestinal tract, the colon bacillus in the lower intestines. The former appears to feed on the carbohydrates, the latter on substances secreted by the intestinal mucous membranes. Only about 10 per cent. of the bacteria seen under the microscope appear as colonies, and whereas in infant stools the majority of the bacteria are frequently Gram positive, the larger number of the colonies are composed of Gram-negative bacteria. Some of the Gram-positive bacteria are anaërobic; others fail to grow on ordinary culture media. These conditions, the normal presence of colon bacilli and the tendency of other bacteria not to grow in culture media, make the greatest care necessary in weighing conclusions as to the pathogenic significance of colon bacilli in disease. Further, although most colon bacilli seem alike in the cruder methods formerly employed, it is now found that there are many differences in their action upon carbohydrates and in their agglutination affinities. There has thus come to be a group of colon bacilli rather than a colon bacillus. Escherich restricts the name to bacilli having the characteristics of those existing in the intestines of normal nursing infants.

The following schematic table illustrates some of the characteristics of members of the colon group and of the typhoid bacilli:

		Motility.	Lactose fermentation.	Milk coagulation.	Indol production.	Flagella.	Growth on potato.	Growth on Uchinsky media.
Bacilli of colon group	A	++	+	+	—	++	++	+
	B	++	+	—	+	++	++	+
	C	+	+	—	—	+	++	+
	D	—	—	+	—	+	++	+
	E	—	—	—	—	+	++	+
Typhoid bacilli		+	—	—	—	++	+	—

The Colon Bacillus under Physiological and Pathological Conditions.—In the breast-fed infant within a few hours or days after birth one or two varieties of typical colon bacilli are found in the colon, and these bacilli form the great majority of all the bacteria present which grow in media. The bacilli in one infant's intestines usually all agglutinate with the same serum, but those from different infants vary. The bacilli find

their way through the food or from the anus upward. In the small intestines the bacterium lactis aërogenes is most prevalent, while in the cæcum and below the colon types predominate.

In *healthy intestines a function of colon bacilli* is supposed to be to prevent, through acids and other products formed, the development of putrefactive bacteria. In normal intestines with intact mucous membranes the toxic products formed by the colon bacilli are absorbed but little or not at all, and the bacilli themselves are prevented from invading the tissues by the epithelial layer and the bactericidal properties of the body fluids. Possibly there is an acquired immunity to the colon varieties which have long inhabited the intestines.

Behavior during Diarrhœa.—In diarrhœa we find increased peristalsis, less absorption of foodstuff, increased and changed intestinal secretions. Tissier observed that under treatment with cathartics the colon varieties increased, while the anaërobic forms are inhibited. In diarrhœa the same conditions are active, inhibiting causes are lessened, and increased mucus and serum are poured out into the canal. This is notably seen in typhoid fever. In diarrhœa, although the common colon varieties are met with, there is usually seen a difference in that uncommon varieties and more typhoid-like bacteria are also found. Much more investigation is needed on this complex subject of variation in types between health and disease.

Passage of Colon Bacilli through the Walls of the Intestines Just Before and After Death.—The colon bacilli tend to pass through the intestinal walls shortly after death in those who have suffered from chronic illness. In chronic disease the resistance of the tissues is lessened or removed, and if to this is added ulceration or loss of epithelium in the lining of the intestines the entrance of the bacteria is still more favored. The temperature at which the body is kept after death determines often whether a general post-mortem invasion takes place. The passage of the colon bacilli before death rarely occurs unless some lesions of the intestines are present.

Varieties of Colon Bacilli as Disease Producers.—The colon bacillus was at first regarded purely as a saprophyte. Later, through not realizing the post-mortem invasion and the great ease of growth of the colon bacillus on ordinary media, the other extreme was taken of attributing too much to it. By means of specific sera and more careful study we are now coming to a clearer view of the action of this micro-organism. The colon varieties met with differ considerably, and we now speak of the colon group rather than of the bacterium coli communis.

It is best to try and separate the cases which come from varieties of the colon bacillus normally present in the intestines from those derived from outside infection. In this latter class the bacilli again vary from the atypical varieties to those classed out of the group, such as the paracolon bacilli, the dysentery bacillus, the bacilli from meat and food infections, etc. These bacilli are considered under their special headings.

The colon bacilli previously in the intestines can, either by an increase in virulence in them or by a lowered resistance in the person,

invade the tissues in which their toxins act, causing injury to the intestinal tract. Thus in the case of ulceration in typhoid fever the colon bacilli enter the blood, or in perforation produce peritonitis. In dying conditions they at times pass through the intact mucous lining. In the gall-bladder or urinary tract the spread of bacilli from the intestines may cause disease. The virulence of the colon bacillus in endogenous infection is usually somewhat increased over that which it possessed when it was latent in the intestine. The specific serum reaction in the body is a sign of infection, but great care has to be observed in deciding that it is present, as group agglutinins also occur. Up to the present time it is very difficult to state in any colon infection whether the bacilli were previously present in the intestines or were derived from outside.

Colon Bacillus in Sepsis.—In lesions of the intestinal mucous membranes or in colon cystitis, pyelitis, or cholecystitis, there is frequently just before death a terminal dissemination of the bacilli and consequent septicæmia. Here special symptoms of intoxication may occur, such as diarrhœa, changes in temperature, heart weakness, and hemorrhages. In most of these cases infection proceeds from the intestines, but in not a few from the wounded urethra or bladder. The colon septicæmia is detected by blood cultures. At times very few bacilli are found, and then the blood infection may be less important than the local one. Cases occurring in typhoid and cholera are often observed, especially in relapses in typhoid. In very young infants a malignant septicæmia with tendency to hemorrhages is due to colon septicæmia. An epidemic due to colon infection of water has been noted. Infection through food and water are usually brought about by other closely allied bacilli not belonging to the colon group.

Colon Bacillus in Diarrhœa.—Lesage, in 1898, stated that 25 per cent. of 770 cases reported by him of breast-fed children were due to pure colon infection, while the others were from mixed infection in which the meaning of the colon bacilli present was more doubtful. The majority of bacteriologists are inclined to doubt that the typical colon bacillus is an important etiological factor in the production of diarrhœa, but believe that it is due rather to other, slightly different, micro-organisms, colon-like in their characteristics. The reasons for this opinion are given by Escherich and Pfaundler as follows:

1. Animals are certainly affected by epidemic infections of bacteria closely allied to the colon group—*e. g.*, diarrhœa of calves and cows, hog-cholera, enteritis with ulceration in horses, etc.

2. The histories of attacks of acute diarrhœa in men after eating food of such infected animals, and the presence of the serum reaction afterward. These bacteria are colon-like, though classed with the enteritidis group.

3. The diseases of typhoidal nature are due to the closely allied paracolon or paratyphoid bacilli, and others are due to the dysentery group, in which the inflammatory and necrotic process localizes itself mostly in the lower colon and rectum.

Numerous epidemics of acute diarrhœa in children from one to five years of age have been noted in which almost pure cultures of colon bacilli have been found. The symptoms begin with high fever which often rapidly falls, and frequent stools containing mucus and streaks of blood or only watery. These symptoms may quickly abate or go on to a toxic state characterized by heart weakness and drowsiness. This may lead to lung complications or death. In many such cases in America when blood has been present we have found one of the mannite fermenting types of the dysentery bacillus. Here the lesions must be considered as being due to mixed infection.

The colon group as exciters of inflammation are much less apt to produce pus than the pyogenic cocci. The peritoneum, the bile tracts, and the urinary tracts are most frequently affected, but it also causes inflammation in wounds and various organs of the body.

Experimental evidence goes to show that the injection of cultures of any of the varieties of colon bacilli into the peritoneal cavity produces intense and fatal peritonitis. Some varieties when freshly isolated are especially virulent. Not only perforation of the intestines in man, but injury to the intestinal walls, allows colon infection of the peritoneum to take place, and if foreign bodies are present in the peritoneum, or the epithelium injured, or absorption interfered with, such acute general peritonitis is very probable. At first most of these cases were believed to be a pure colon infection, but now it is known that this idea came largely from the overgrowth of colon bacilli in the cultures. More careful investigations, through cultures and smears, have demonstrated the fact that streptococci, and less frequently staphylococci and pneumococci, are present in peritonitis arising from intestinal sources. The colon bacilli found even in the same case commonly comprise many varieties.

The Colon Group in Inflammation of the Bile Tract.—The contents of the gall-bladder are usually sterile. This is true in spite of the fact that bile is apparently a good culture medium for the colon group. Simple tying of the neck of the gall-bladder usually causes a colon infection to take place within twenty-four hours. Obstruction of the bile-duct through various causes is fairly common in man. The gall-bladder then becomes infected, and following the inflammation of the mucous membranes there is often the formation of gallstones. Some cases of jaundice are believed to be due to colon inflammation of the gall-ducts.

Inflammation of the Pancreas.—Welch was the first to record a case of pancreatitis with multiple fat necroses due to colon infection. A few more cases have since been reported as due to members of the colon group, either alone or in conjunction with the pyogenic cocci.

Inflammation of the Urinary Tract.—As far back as 1879 Bouchard noted cystitis due to bacilli of the colon group. After injury of the bladder mucous membrane, or by ligature of the urethra, it is possible to excite cystitis in animals by injection of colon bacilli. When cystitis is established the bacterial infection frequently spreads to the pelvis of the kidneys, causing a pyelitis or suppurative nephritis. The same

process often occurs in man. In most cases of chronic cystitis the ureters and pelves of the kidneys become involved; any malformation of the ureters aids the process. From the pelvis the bacteria push up into the urinary tubules and excite inflammation and multiple abscesses. At times the bacilli force their way through the lymph channels or capillaries into the blood current. Colon infection of the different parts of the urinary tract may occur at any age, from infancy upward. Instead of starting in the bladder it may begin in the kidney itself, the colon bacilli coming from the blood or peritoneum. In many of these cases the bacilli isolated from the urine are clumped in high dilutions of blood from the patient.

Although other bacteria—the pyogenic cocci, the proteus, the typhoid bacillus, etc.—may excite cystitis, still in 90 per cent. of all cases some of the colon group are found, and this percentage is even higher in young children. The clinical picture of colon infection is very variable. The lightest cases progress under the guise of a bacteriuria. The urine is passed a little more frequently and shows a fine granular cloudiness. The reaction is acid. The cell elements are but little increased. There is an excess of mucus. Albumin is absent or present in only a trace. The condition may last for weeks or months and then spontaneously disappear or grow worse. With a somewhat more severe infection there is painful urination, perhaps tenesmus, increase of pus cells, and slight fever. In a conical glass a sediment of pus cells forms at the bottom, and clear urine remains above. In chronic cases the fever is usually absent, but anæmia and loss of tone appear. If the infection passes to the kidney colicky pain and tenderness over the region of the kidneys are usually present. The most important symptom of pyelitis is an irregular intermittent fever resembling malaria. The albumin is increased in the urine and red blood cells may be seen. If a general nephritis arises the symptoms are all intensified and an anæmic condition may develop. A septicæmia may finally result. Therapeutic bacteriological means are employed to treat this affection; irrigation of the bladder with creolin, lysol, and silver solution. Internally urotropin or salol are given.

In most of these cases the microscopic examination is sufficient to make a probable diagnosis, since the bacteria are so abundant. The variety of colon bacillus present can, of course, only be told by cultures and other means. In the urine they appear as diplobacilli, or partly in short, almost coccus, forms, partly in long threads. As a rule, motility is absent. Not infrequently the cultures appear to be identical with the bacterium lactis aërogenes.

The characteristics of the urine itself have much to do with the probability of infection; the more acid urines being less likely to afford a proper soil for growth. Some urines are bactericidal even when they are neutral. The substances producing this condition are not known. The colon bacilli in the urine produce no appreciable effect on the reaction, but give up some of their toxins, which upon absorption cause the deleterious local and general effects. The serum of the

patient usually agglutinates the cultures from the urine in 1:20 or 1:50 dilutions, but this property is sometimes absent, especially in light cases.

The colon variety found in the urine can ordinarily be detected also in the feces. This would suggest an autoinfection. Cystic infection in the female usually takes place through the urethra. In both male and female any injury or disease of the rectal mucous membranes contiguous to the bladder creates a possibility of the invasion of the colon through the lymph channel of the inflamed tissues and cause a colon cystitis. It is also possible that at times an infection may take place through the kidney, this organ having received the bacilli from the blood. Besides the above-mentioned ways, we may have direct infection carried in by the catheter, sound, etc.

In all cases in addition to the introduction of the colon bacillus a predisposing condition must be present, such as more or less marked retention of urine by an enlarged prostate or stricture, any unhealthy state of the mucous membrane or general depression of vitality.

The Colon Group as Pus Formers.—Members of this group are frequently the cause of abscesses in the region of the rectum, urethra, and kidney. They are rarely met with in other locations.

The Colon Group in Inflammation Not Previously Mentioned.—Bronchopneumonia, lobar pneumonia, and pleurisy have occasionally been caused by colon bacilli, probably from blood sources. Not a few cases of meningitis and spinal meningitis in infants, following localized colon infections, are due to colon bacilli. The symptoms are not well developed, as a rule. Some cases of endocarditis have also been noted.

The Colon Group as Producers of Absorbable Toxins.—Through intestinal fermentation substances are formed which when absorbed produce marked nervous symptoms. The presence of indican in the urine usually means that such improper fermentation is present.

Methods of Isolation.—While the isolation of typhoid bacilli from feces, water, dust, etc., is attended, as a rule, with difficulty, pure cultures of colon bacilli can usually be obtained from such substances by the simplest procedures. The following methods may be recommended:

1. Inoculate 10 c.c. of fluid 2 per cent. lactose or dextrose litmus agar with feces or suspected material. The melted agar should be at a temperature of about 41° C. After shaking pour in Petri dish. Several dilutions should be made. After eighteen hours examine the plates and inoculate the contents of a number of tubes containing 2 per cent. dextrose agar with any colonies showing a red color. The colon bacilli will produce gas and acid.

2. Inoculation of increasing quantities of the material (water) in 2 per cent. dextrose nutrient bouillon. The presence of colon bacilli in the inoculated portion produces after twelve to twenty-four hours active fermentation in the tube. (Plate and isolate as in 1.)

3. The inoculation of glucose bouillon to which small amounts of carbolic acid or of hydrochloric acid have been added to inhibit the growth of many other varieties of bacteria. This method has not given any better results than 2.

Intermediate Members of the Typhoid-colon Group of Bacilli.

Until recent years the difficulties attending the differentiation of the various members of the typhoid and colon groups of bacilli have been so great that an accurate study of the varieties has not been possible. The application of the Gruber-Widal reaction to this investigation has, however, served to clear up the field to a great extent, so that at the present time the differentiation is comparatively easy. Gärtner's discovery of the bacillus enteritidis in 1888 in association with epidemics of meat poisoning first gave impetus to the study of the intermediates. Nocard's work on bacillus psittacosis followed in 1892. In 1893 Gilbert introduced the terms "paracolon" and "paratyphoid" to designate bacilli of this group resembling more nearly in biological characters the colon bacillus on the one hand and the typhoid bacillus on the other, but at that time the organisms now known as paratyphoid bacilli had not been identified.

The intermediates include bacillus enteritidis and similar organisms recovered from cases of epidemic meat poisoning, the gas-producing typhoid-like bacilli of various observers, bacillus psittacosis, bacillus choleræ suis, bacillus icteroides, bacillus of calf septicæmia, and the various paratyphoid and paracolon bacilli that have been described recently.

The bacilli intermediate between bacillus coli communis and bacillus typhosus can be distinguished without difficulty from either of them. They produce gas in glucose media and in this respect they differ from typhoid, but, unlike bacillus coli communis, they have no power of fermenting lactose, coagulating milk or forming indol.

They are not agglutinated by typhoid sera except, as in the case of colon bacilli, imperfectly in low dilutions because of group agglutinin.

Among the intermediates themselves, however, two main groups can be recognized, and it is proposed to call these *paracolon* and *paratyphoid* groups, the former appearing in some respects to be more nearly allied to bacillus coli communis and the latter more nearly to typhoid.

The main points of difference are that the paracolons turn milk and whey alkaline after a short initial acidity and form gas freely in glucose media, while with the paratyphoids there is in milk and whey an initial acidity, but no or very slight subsequent alkalinity; the gas production in glucose media is much less pronounced. Neutral red agar also differentiates between the two groups. Like bacillus coli communis all the intermediates reduce the color to yellow in twenty-four to forty-eight hours, but with the paratyphoids after four or five days the red color begins to return from above downward until in two or three weeks the medium is again red throughout. With the paracolons the yellow color is permanent.

Agglutination tests have taught us that the members of the coli communis group do not constitute a distinct species as in the case with typhoid bacilli. When these tests are applied to the intermediates it is found that the members of the paracolon group do not all show mutual

reactions, and the group must, therefore, be composed of a number of distinct races, as is the case with bacillus coli communis. The paratyphoids, on the other hand, none of which has so far been isolated from cases other than typhoidal, interact without exception; that is to say, an active serum prepared from any one of the bacilli will agglutinate all the members of the group. The paratyphoids, then, appear to be a distinct species in the same sense that the typhoid bacilli are a distinct species.

It may be found possible to distinguish various species within the paracolon group. There are certainly three members which afford mutual reactions—one of those isolated by Schottmüller, one by Kurth, and one by Libman—and these might provisionally be called β-paratyphoids. Since, however, there are others of the paracolon group which have caused typhoidal symptoms, yet do not appear to belong to this particular species, it seems a little premature to attempt these fine distinctions, and for the present it will be sufficient to confine ourselves to the idea of the two main groups:

I. *The Paracolons.*—A group of bacilli, the members of which are culturally alike, but constitute several distinct species, some of which may give rise to typhoidal symptoms in man.

II. *The Paratyphoids.*—A distinct species, culturally unlike the paracolon bacilli, and causes typhoidal symptoms in man.

Pathogenicity in man has been established for other of the intermediates. Broadly speaking there are three types of infection:

1. Typhoid type, caused by the paratyphoid bacilli and certain of the paracolons.[1]

2. Epidemic meat-poisoning type, caused by bacillus enteritidis and its allies.

3. Psittacosis type, caused by bacillus psittacosis.

In addition to these three types Grünbaum has suggested that febrile jaundice may be caused by one of the intermediates.

Bacillus icteroides (Sanarelli), which is probably an intermediate, is no longer the accredited cause of yellow fever, but is considered simply as a secondary invader. It is stated that it ferments dextrose and saccharose.

The Typhoid Type. *The Term Paratyphoid Fever.*—Though Gilbert introduced the terms "paracolon" and "paratyphoid" in 1893 to designate groups of bacilli, Achard and Bensaude (1896) were the first to employ the term paratyphoid in a clinical sense. This use of the term was sanctioned by Schottmüller in 1901, and has been adopted by several recent writers. On the other hand, Coleman seriously questions whether the term should be recommended, as it leads to unfortunate multiplicity. He considers it proven that infection with paratyphoid bacilli is often manifested by symptoms practically identical with typhoid fever except for the Widal reaction, that there are differences (biological and in serum reactions) even among the paratyphoid bacilli themselves (β-paratyphoids), and that bacilli of the enteritidis

[1] Petruschky's bacillus fæcalis alcaligenus, while not an intermediate of the group (Durham), may also produce typhoidal symptoms.

type may at times produce typhoidal symptoms. He believes it is no more advisable to make a clinical subdivision of these cases than of the cases of pneumonia or infective endocarditis which may be due to one of several different micro-organisms. Paratyphoid infections do not constitute a clinical entity. There is at least as great diversity among the different types of typhoid fever as between typhoid fever and paratyphoid infections. Moreover, typhoidal symptoms may be produced by Petruschky's bacillus fæcalis alcaligenus (the author states that it was obtained from the feces of patients suspected to have typhoid fever) and yet this bacillus is not an intermediate. It lies just without the group on the typhoid side, in that it does not acidify any sugar-containing medium (Dunham). It is true that the intestinal lesions are different in that Peyer's patches are not usually markedly involved. Even when fatal hemorrhages occur there are usually found only one or more deep erosions. The average course is milder and the method of infection somewhat different.

These various considerations make it necessary to abandon the idea of the *specificity* of the clinical disease typhoid fever. As in the case of abscesses the physician recognizes the clinical fact, the bacteriologist determines the causative agent. It certainly seems better to confine the terms "paratyphoid" and "paracolon" to the domain of bacteriology and to hospital practice, where bacteriological examinations can be carefully made, and to broaden the scope of the etiology of typhoid fever to include these several organisms—bacillus fæcalis alcaligenus (?), bacillus typhosus, bacillus paratyphosus, and certain members of the paracolon group (β-paratyphoids).

Geographical Distribution and Relative Frequency of Paratyphoid Infection.—The cases have been widely distributed geographically, having occurred in Paris, Hamburg, Bremen, Strassburg, Liverpool, Philippine Islands, New York City, Baltimore, and Philadelphia.

Very little can be said of the relative frequency of paratyphoid infections. Gwyn's case was the only one of 265 cases which failed to give the Widal reaction. Six of Schottmüller's cases occurred in a series of 68 and Kurth's 5 in a series of 62 cases whose sera were tested for the Widal reaction. Johnston's 4 cases were found among 194, and Hewlett's 1 in a series of 26 cases of typhoid fever. Hünermann has reported an epidemic of 38 cases of paratyphoid infection occurring in the garrison at Saarbrück. Falcioni reports 5 cases out of 100 cases of supposed typhoid fever. The proportion of negative Widal reactions is low in the statistics, but there is a source of error here in that until very recently the tests have not been made in high-enough dilutions—that is, at least as high as 1:40.

Post-mortem Findings.—Autopsies were performed on 3 fatal cases (Strong, Longcope, Tuttle). The interest in these autopsies naturally centres on the condition of the intestine. Strong states that both the large and small intestines were normal throughout except for moderate catarrh and a few superficial hemorrhages. The solitary and agminated follicles showed no lesions. The mesenteric lymphatics, how-

ever, and some along the small intestine, were hemorrhagic. In Longcope's case the intestine showed no changes either on gross or microscopic examination. The spleen in both cases was enlarged. The other pathological changes were those common to febrile conditions. In Tuttle's case a few erosions just above the ileocæcal valve were present.

SOURCE OF INFECTING BACILLI.—Tuttle's case happened to be a laboratory employé in the service of the Department of Health and was carefully investigated by us. We found that two families consisting of eleven members drank water from an open uncovered tank. During the summer the tank was not cleaned and was only occasionally filled by pumping in water from the city supply. Sometimes the water was the color of tea. During a single week four members of one family and three of the other were stricken with a typhoid-like fever. The two families had no social intercourse with each other.

Symptomatology.—It is a significant fact that many of the reported cases of paratyphoid infection were considered to be genuine typhoid fever without the Gruber-Widal reaction until a bacteriological study revealed their true nature.

Intestinal hemorrhages, furunculosis, initial bronchitis, cystitis, pyelonephritis(?), purulent arthritis, bronchopneumonia, and venous thrombosis have been reported as complications. Osteomyelitis is the only recorded sequel.

The duration of the disease has varied from twelve to eighty-four days, with a majority of the cases continuing between twenty and thirty-six days. Some of the cases have been of short duration, lasting from twelve to eighteen days.

The Serum Reaction in Cases of Paratyphoid Infection.—Since the introduction of serum reactions as a means of diagnosis, it has been a well-recognized fact that a small proportion of cases which are clinically typhoid fever fail to give the reaction. Brill, adding to Cabot's statistics, finds that of 4879 cases 4781, or 97.9 per cent., gave the reaction. Gwyn gives 99.6 per cent. as the percentage of positive reactions in the Johns Hopkins Hospital. On the contrary, in most of the reported cases of paratyphoid infection a reaction, except with low dilutions, against the bacillus typhosus has been absent. It is probable, then, that some at least of the typhoid cases with negative reaction were really paratyphoid infection.

On the other hand, it cannot be assumed that all cases clinically typhoid fever, which have been reported as giving the Gruber-Widal reaction, were cases of genuine typhoid infection. The brilliant work of Dunham on the typhoid-colon group of bacilli and its serum reactions has brought out the fact that certain members of this group may be mutually interacted upon by sera of infected patients and of immunized animals. This is especially true of sera in low dilution. Since in the earlier years of the Gruber-Widal reaction the technique had not been worked out, and dilutions were more frequently low than not, some of the cases reported as typhoid fever may have been infections with paratyphoid bacilli.

Diagnosis.—The only reliable criteria for diagnosis are absence of the Gruber-Widal reaction in proper dilution (not less than 1:40) with a positive reaction against a known paratyphoid bacillus or the recovery of a paratyphoid bacillus from the blood, urine, or complicating inflammatory process.

The clinical type of the disease is of little value in a single case. It has already been stated that the reported cases of paratyphoid infection have been both mild and severe.

The cases of paratyphoid infection are too few to state what the *prognosis* should be. It can only be said that the majority of the cases have been mild, though there have been about 9 per cent. of deaths among the cases reported.

EPIDEMIC MEAT-POISONING TYPE.—Gärtner announced his discovery of bacillus enteritidis as the cause of epidemic meat poisoning in 1888. A cow sick for two days with profuse diarrhœa had been slaughtered in Saxony and the meat sold for food. Of the persons who ate of the meat 57 became ill, and 1 died. Gärtner recovered the bacillus from the meat and from the organs in the fatal case.

Previous to Gärtner's discovery the cause of meat poisoning had been held to be bacterial products, and while this may still be true in certain instances there is no satisfactory evidence to support the contention. All cases in which a thorough bacteriological examination has not been made must be excluded.

Two kinds of bacilli are concerned in the production of meat poisoning: 1. Anaërobic bacillus botulinus of Van Erminghem, a saprophyte. 2. Bacillus enteritidis of Gärtner, including the different strains of this organism.

Of these bacilli, bacillus enteritidis is the more important, having been concerned in the greater number of epidemics, and causing true meat poisoning. It seems advisable, however, to say a few words, by way of distinction, on infection by bacillus botulinus.

"Botulism," "allantiasis," and "sausage poisoning" are the names given to infection by bacillus botulinus. The infection of the meat takes place after the animal has been slaughtered. The meat is of unsound appearance and odor and can readily be seen to be unfit for food.

The symptoms begin from twelve to twenty-four hours after ingestion of the meat, with repeated attacks of vomiting and abdominal pain. Soon the characteristic symptoms appear: partial or complete paralysis of the inner and outer recti muscles of the eye, and disturbances of the innervation of the pharynx and larynx. These are manifested by imperfect vision, difficulty of speech and deglutition, and dryness of the throat. There are no disturbances of sensation or impairment of consciousness and the disease runs its course without fever. Constipation and retention of urine follow; dyspnœa and cardiac failure appear, and bulbar paralysis may supervene, causing death. In earlier years the mortality from sausage poisoning was from 30 per cent. to 50 per cent., but this has been much reduced through a better

understanding of the disease. It may be prevented by thoroughly cooking the meat and by refusing to accept from the butcher meat that is the least tainted.

True Meat Poisoning.—This form of meat poisoning is due to bacillus enteritidis, and in every instance the animal is diseased at the time of the slaughter. It may be contracted by eating sausage, since the meat of diseased animals is sometimes surreptitiously put on the market in the form of sausage.

Dunham makes bacillus enteritidis the chief type of the intermediates and proposes the name "the enteritidis group." Buxton classes the bacillus with the paracolons. It does not ferment lactose; milk becomes more alkaline; it ferments dextrose with a production of gas containing about one-third CO_2, two-thirds H, and it also ferments mannite, maltose, and dextrin.

Bacillus enteritidis is pathogenic for cows, horses, pigs, goats, mice, and guinea-pigs, but not for dogs and cats.

The Infected Meat.—In many epidemics bacillus enteritidis has been isolated, not only from the organs of fatal cases, but from the suspected meat. The meat does not differ in appearance or taste from that of healthy animals. It has already been stated that it may be made into sausages, and one epidemic at least has been caused by eating "dried meat" consisting of large pieces of the flesh of sheep and goats nearly dried in the sun and eaten cooked or merely softened by soaking. Cooking does not always destroy the bacilli, as the thermal death point may not be reached in the interior of the meat. Infected meat which is not eaten immediately after it has been cooked is especially dangerous.

The meat has always come from animals sick at the time of slaughter. The meat of cows and calves have most often caused the epidemics, though that of horses, pigs, and goats have also been responsible. Dunham says that no known case has come from mutton, and that the pig has been implicated in only one outbreak which has been studied bacteriologically. In this connection it is interesting to recall that Theobald Smith has insisted on the similarity between the hog-cholera bacillus and bacillus enteritidis.

The animals from which the infected meat has come have suffered during life from puerperal fever and uterine inflammations, navel infection in calves, septicæmia, septicopyæmia, diarrhœa, and local suppurations, and have not infrequently been killed because of their unsound condition. How animals become infected is not known.

Dunham thinks milk may be a source of infection in man, but states that bacteriological evidence of it is incomplete. Bacillus enteritidis has been found, however, in the milk of infected guinea-pigs (Basenau).

Transmission to Man.—The disease may be transmitted to man in two ways: (1) by eating the infected meat, and this is by far the most common means, and (2) from man to man according to Gärtner, Van Erminghem, and Fischer. Dunham found inconclusive evidence of this

means of transmission in one epidemic. Fischer thinks transmission may take place through the excreta. It will subsequently be seen that psittacosis may be transmitted from man to man.

Epidemics of meat poisoning may occur in any season, but are more frequent during the warm months.

Symptomatology.—While the characteristic symptoms of sausage poisoning relate to the nervous system, in true meat poisoning they are gastrointestinal. Fischer divides meat poisoning into three clinical forms: (1) typhoidal; (2) choleraic; (3) gastroenteric.

Prevention.—Since neither appearance nor taste affords any clue to the noxious quality of the infected meat, its unfitness for food can only be told through bacteriological examination or a knowledge of its source. Dunham states that thorough cooking will kill the bacilli, but it must be remembered that in this process the thermal death point of the bacilli may not be reached in the innermost portions of the meat.

Bacillus Fæcalis Alcaligenus.

This bacillus resembles a colon bacilus which has lost its power to ferment sugars. Morphologically it resembles the typhoid bacillus. It is frequently present in the intestines and may have pathogenic properties.

The Dysentery Bacillus—The Paradysentery Bacilli (Mannite Fermenting Types).

Dysentery may be divided into acute and chronic. Amœbæ appear to be the chief exciting factor in most cases of chronic dysentery, though bacilli of the colon group also play a part.

In temperate climates acute dysentery is but very rarely due to amœbæ, but usually to the bacilli identified by Shiga or to allied bacilli identified by Kruse, Flexner, and Park. By dysentery we mean a definite symptom complex; it is not an etiological term. In acute dysentery the onset is sudden and ushered in by cramps, diarrhœa, and tenesmus. The stools at first feculent, then seromucous, become bloody or composed of coffee-ground sediment. At the height of the disease there are ten to fifty stools in the twenty-four hours. After two to seven days the blood usually disappears. In temperate climates the mortality varies from 5 to 20 per cent.

In severe cases in adults the lesions are of a diphtheritic character and may be very marked. In young children, even in fatal cases, the lesions may be more superficial. The following macroscopic and microscopic report of the intestinal findings on a fatal case of bacillary dysentery in an infant is a typical picture:

Small Intestines.—Slightly distended. Mesenteric glands large and red.

Large Intestines.—Outer surface vessels congested and prominent. On section covered with a yellowish mucus. Mucous membrane seems

to be absent in places. Solitary follicles are elevated and enlarged, especially in the region of sigmoid flexure. In some instances the centre of the follicles are depressed and appear to be necrotic.

Appendix.—On section lymphatic follicles are swollen with depressed centres similar to the condition described in the large intestines.

Small Intestines.—Peyer's patches are distinctly swollen, but in no instance is there ulceration or necrosis.

Large Intestine.—Mucous glands are for the most part normal, but over the solitary follicles they have broken down somewhat and contain polynuclear leukocytes. The interglandular stroma in these places has undergone necrosis. The necrotic area extends down into the submucosa in the region of the solitary follicles. The capillaries of the solitary follicles are much dilated and congested. The submucosa is thickened and slightly œdematous. The connective-tissue cells appear to have undergone a slight hyaline degeneration. The musculature is not affected, neither is the peritoneal coat.

Small Intestines.—Normal.

Historical Notes.—In 1897 Shiga found in the stools of cases of dysentery a bacillus which had not been before identified. This bacillus had many of the characteristics of the colon bacillus, but differed from it, lacking motility and failing to produce gas from the fermentation of sugar. It also was entirely distinct in its agglutination characteristics and in its pathogenic properties. Shiga found this bacillus present in all the cases of epidemic dysentery that he examined. It was most abundant during the height of the disease and disappeared with the return of fecal stools. It was not found in the stools of healthy persons. He found that the blood of dysenteric patients contained substances which agglutinated the bacilli that he had isolated. The serum from healthy individuals did not agglutinate the bacilli to any such degree as the serum from those sick with dysentery. When the mucous membrane of the colon was examined in fatal cases dying in the height of the disease, the bacilli were found in the superficial layers in almost pure cultures. In his hands a serum produced by immunizing horses through injections of dysentery bacilli gave beneficial results when used in the treatment of those ill with the disease. A criminal fed with a culture of the bacillus developed typical dysentery. Certain animals, such as dogs, when subjected to treatment which made them more susceptible, were attacked with dysentery after feeding on cultures. This was fairly similar to that in man.

Morphological and Cultural Characteristics of Dysentery Bacilli. MICROSCOPIC.—Similar to bacilli of the colon group.

STAINING.—Similar to bacilli of the colon group.

MOTILITY.—No definite motility has been observed. The molecular movement is very active.

FLAGELLA.—True flagella have not been observed by most examiners. On a very few bacilli in suitable smears Goodwin demonstrated terminal flagella. Spores are not formed.

Appearance of Cultures.—On gelatin the colonies appear more like the typhoid than the colon bacilli. Gelatin is not liquefied. On agar growth is somewhat more delicate than the average colon cultures.

On Potato.—A delicate growth just visible or distinctly brownish.

In Bouillon.—Diffuse cloudiness with slight deposit and sometimes a pellicle. Indol not produced or in a trace only.

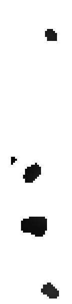
In glucose bouillon no production of acid or gas.

Neutral red agar is not blanched.

In Litmus Milk.—After twenty-four to forty-eight hours this becomes a pale lilac. Later, three to eight days, there is a return to the original pale blue color. The milk is not otherwise altered in appearance.

Animal Tests.—No characteristic lesions have followed swallowing large quantities of bacilli. Dogs at times have had diarrhœa with slimy stools, but section showed merely a hyperæmia of the small intestine.

Fig. 86



Dysentery bacillus.

Fig. 87

Colony of dysentery bacilli in gelatin.

Many animals are very sensitive to bacilli injected in vein or peritoneum; 0.1 mg. of agar culture injected intravenously produced diarrhœa, paralysis, and death; 0.2 mg. under the skin have killed, and the same amount in the peritoneum has caused bloody peritonitis, with lowered temperature and diarrhœa. Both small and large animals are very sensitive to killed cultures.

The autopsy of animals dying quickly from injection into the peritoneum of living or dead bacilli shows the peritoneum to be hyperæmic, the cavity more or less filled with serous or bloody serous exudate. The liver is frequently covered with fibrinous masses. The spleen is moderately or not at all swollen. The small intestine is filled with fluid, the large intestine is usually empty. The mucous membrane of both is hyperæmic and sometimes contains hemorrhages. Conradi found ulcer formation in one case.

Subcutaneous injections of dead or living cultures are followed by infiltration of tissues and frequently by abscess formation. The dysentery bacilli produce both extracellular and cellular toxins, the latter being the most abundant. The elimination of these toxins from

the body is supposed to take place through the intestines, and this gives rise to the intestinal lesions in animals injected intravenously or intraperitoneally. The dysentery bacilli are not found in the blood or organs of animals.

Paradysentery Bacilli as Exciters of Dysentery.—In 1900 Flexner and Strong, when in the Philippine Islands, isolated bacilli from dysenteric stools which were identical with the Shiga cultures. At first all the cultures were supposed to be of the Shiga type, but later among those isolated bacilli were found, which differed from Shiga's in many characteristics. In the same year Kruse, in Germany, obtained from dysenteric cases in an asylum bacilli which appeared to him to be culturally like those isolated by Shiga, but to differ in their agglutinating characteristics. These, like those isolated by Flexner, were later found to differ in many characteristics. In 1902 Duval and Bassett, in Baltimore, thought they had found the Shiga bacilli in the stools of a number of cases of summer diarrhœa. These later proved to be identical with some of the bacilli isolated by Flexner in Manila. During the same summer Park and Dunham isolated a bacillus from a severe case of dysentery occurring during an epidemic at Seal Harbor, Mt. Desert, Maine, which they showed to differ from the Shiga bacillus in that it produced indol in peptone solution and differed in agglutinating characteristics.[1] They at first considered it identical with the Philippine culture given them by Flexner, but in January, 1903, it was shown by Park to be a distinct variety, and later found by him to be the exciting factor in a large number of cases.

Martini and Lentz[2] published the results of their work in December, 1902. They showed that the Shiga type of bacilli obtained from several separate epidemics in Europe agreed with the original Shiga culture in that it did not ferment mannite. The cultures of this type agreed with each other in agglutinating characteristics. When the bacilli from Flexner, Strong, Kruse, Park, Duval and others, which differed from the Shiga culture in their agglutinins, were tested they were all found to ferment mannite. Martini and Lentz considered that the Shiga bacillus was the true dysentery type and that the mannite fermenting variety or varieties might be mere saprophytes, or perhaps be a factor in the less characteristic cases.

In January, 1903, Hiss[3] and Russell, independently of others, showed that a bacillus isolated by them from a dysenteric stool differed from Shiga's bacillus in the same characteristics as mentioned by Martini and Lentz.

At the beginning of the summer of 1903, therefore, it was established, although not fully recognized, that there were in dysenteric stools at least two distinct types of bacilli, the true Shiga type and the type fermenting mannite and producing indol. It had also been established that the second type contained more than one variety.

[1] New York University Bulletin of the Medical Sciences, October, 1902, p. 187.
[2] Zeitschrift f. Hygiene u. Infectionskrank., 1902, xli., 540 and 559.
[3] Medical News, 1903, lxxxii., 289.

The German observers considered the Shiga type as the only one which had established its causal relation to acute dysentery, while the American observers generally considered both types to have equal standing and some[1] of them considered these differences as not important and perhaps not permanent. This latter opinion seems to have been held by Shiga.[2]

We took up the investigation at this point with the object of carefully studying the bacilli isolated by us from acute dysentery, which occurred in a number of widely separated epidemics. We hoped thus to determine whether the bacilli exciting acute dysentery in the Eastern States belonged to a few distinct types or were divided into a large number of varieties.

In the most extensive epidemic that has recently occurred in the region of New York City there were in all some 500 cases of acute typical dysentery. Whole families were attacked with the disease.

The majority of the cases were of moderate severity, the dysenteric discharges lasting from one to two weeks. There were a number of light cases, but all had dysenteric stools containing mucus and blood. The mortality was about 6 per cent. Judging from the cases investigated by us, over one-half of those attacked seem to have been infected by the Shiga type, and these were, as a rule, the most severe cases. Most of the cases in two severe, though localized, epidemics in a Pennsylvania town and at Sheepshead Bay were also due to this type. The mortality was higher in these epidemics. The facts published abroad also indicate that this variety has been found in the chief epidemics in Europe and Asia. The bacilli isolated in the severe epidemic of dysentery reported by Vedder and Duval (at New Haven, Conn.) were chiefly of this type. We have never yet succeeded in isolating bacilli which had all the characteristics of the Shiga variety from any diarrhœa cases in which no dysenteric symptoms appeared.

We turn now to the mannite fermenting varieties, whose relationship to dysentery is still doubted by some.

The cultures isolated by us from over 40 cases were found to fall largely into two distinct types, one of which differs from the Shiga bacillus more radically than the other.

The variety nearer to the Shiga bacillus has the characteristics of the culture, which was isolated by us at Seal Harbor, Maine, in August, 1902. The other variety is represented by the Flexner Philippine type.

The first type differs from the Shiga bacillus in its agglutinating characteristics and in that it produces considerable indol in peptone solution and ferments mannite with the production of acids. The second type differs in these points and in addition that in its agglutinating characteristics ferments saccharose and chemically pure maltose in peptone solution.

[1] University of Pennsylvania Medical Bulletin, July and August, 1903.

[2] Zeitschrift f. Hygiene u. Infectionskrank., 1902, xli., 356.

Besides the epidemic at Seal Harbor, numerous cases of moderately severe or slight dysentery due to the first type were met with in the extensive epidemic which has been already alluded to in the towns north of New York City. A few characteristics in many slightly developed cases of dysentery in New York City during the past two summers were caused by this type of bacillus. A great many cases are also due to the Philippine type. A number of rather severe cases of dysentery developed in Orange, N. J. Cultures from two cases were made, and this type alone obtained. The following is a typical case. Eighteen out of thirty colonies, selected from the plates, when tested proved to be dysentery bacilli of the Philippine type.

Dorothy D., aged two years and three months. Seen first July 29th, a day after the child had eaten green apples. Previous to this the child had had an attack of vomiting and diarrhœa, the sickness lasting two weeks; the diarrhœa had subsided only two days before present illness. No blood was seen during this first attack. When first seen, the child had a temperature of 104.6°, with vomiting and diarrhœa. After a calomel purge the patient was better; the following day, however, the diarrhœa started up again and the temperature rose. The stools were numerous, small, containing mucus and blood, preceded by pain and accompanied by tenesmus. Many of the stools consisted of nothing but blood and mucus. Sixteen movements were the greatest number recorded in a day. On August 2d ten cubic centimetres of dysenteric serum were injected. There seemed to be some improvement in the character of the stools following the injection. On August 4th and 6th the injections were repeated, being followed each time, apparently, by some improvement in the child's condition. The blood disappeared in eight or nine days, and the child had then five movements daily, consisting largely of mucus.

At Riker's Island a number of men were filling in new land. Dysentery broke out and spread to a number of the men, as well as to the physician in charge. Those infected had usually a short, sharp attack with a quick recovery. Very large amounts of blood were passed by some of the sick.

In some a large proportion of the bacteria isolated were bacilli of the Philippine type. No other type of dysentery bacilli was found in any of the cases in this epidemic.

Charlton and Jehle report a series of cases occurring in St. Ann's Hospital, Vienna, in which mannite fermenting types were the only dysenteric-like organisms present. The cases on the average ran a much milder course than those in which the true dysentery bacilli were present.

When the agglutinating characteristics of these bacilli and their susceptibility to immune sera are studied carefully, we find that each of the three types differs from the others. The mannite and the maltose types, since in animals they stimulate abundant common agglutinins and immune bodies, seem more closely allied to each other than to the Shiga type.

This is seen in the following tables, in which bacilli from a number of cases obtained from different sources are tested in sera from animals which have each received a single type of dysentery bacillus:

TABLE I.—*Agglutination of bacilli belonging to the three types in the serum of a young goat injected with the bacillus isolated by Shiga, in Japan.*

	Dilutions of Serum.						
Source.	1 : 20	1 : 50	1 : 100	1 : 200	1 : 500	1 : 2000	1 : 5000
Type I. Shiga.							
1. Original, Japan—Shiga,	++	++	++	++	++	++	+
2. New Haven—Duval,	++	++	++	++	++	++	+
3. Tuckahoe—Carey,	+ |	++	++	++	++	+ |	±
4. Coney Island—Collins,	++	++	++	++	++	++	+
5. Mt. Vernon, Case I.—Collins,	++	++	++	++	++	+ |	+
6. " " " II. "	++	++	++	++	++	++	±
7. " " " III. "	++	++	++	++	++	+	±
Type II.							
8. Original, Mt. Desert—Park,	+ |	+ |	|	—	—		
9. New York City—Goodwin,	+ |	+ |	|	—	—		
10. Hospital, New York—Collins,	+ |	+ |	|	—	—		
11. Foundling Hospital—Hiss,	+ |	+ |	|	—	—		
12. Mt. Vernon, Case I.—Collins,	+ |	|	—	—	—		
13. " " " II. "	++	+ |	—	—	—		
Type III.							
14. Original, Manila—Flexner,	+	+	+	±	—		
15. Baltimore—Duval,	+ |	+ |	+	±	—		
16. New York City—Wollstein,	++	+	±	|	—		
17. Orange—Collins,	+	+	±	—	—		
18. Riker's Island—Goodwin,	++	++	+	|	—		

The serum of this goat before injection did not agglutinate any of the above bacilli in 1 : 10 dilution.
++ = complete reaction. + = good reaction. | = slight reaction.
+ | = very good reaction. ± = fair reaction. — = no reaction.

TABLE II.—*Showing agglutination of members of three types in the serum of animals injected with bacilli of Type II.*

	Goat injected with No. 4.					Rabbit injected with No. 6.				
Source.	1 : 20	1 : 50	1 : 100	1 : 500	1 : 1000	1 : 20	1 : 50	1 : 100	1 : 500	1 : 800
Type I. Shiga.										
1. Japan—Shiga,	+	—	—	—	—	|	—	—	—	—
2. New Haven—Duval,	+	—	—	—	—	|	—	—	—	—
3. Tuckahoe—Carey,	+	—	—	—	—	|	—	—	—	—
Type II.										
4. Mt. Desert—Park,	++	++	++	++	++	++	++	++	+	|
5. Mt. Vernon—Collins,	++	++	++	++	++	++	++	++	+	+
6. New York—Hiss,	++	++	++	++	++	++	++	++	+	—
Type III.										
7. Manila—Flexner,	++	++	+	—	—	++	++	++	—	—
8. Baltimore—Duval,	++	++	+	—	—	++	++	++	—	—
9. Riker's—Goodwin,	++	++	+	—	—	++	++	++	—	—

The serum of the above animals previous to immunization did not agglutinate any of the above bacilli in a 1 : 20 dilution.

TABLE III.—*Showing agglutinations of members of three types in the serum of animals injected with bacilli of Type III.*

	Rabbit injected with Baltimore, Duval.						
	10	50	100	500	1000	5000	10,000
Type I.							
1. Japan—Shiga, and 5 other cultures,	++	+	+	—	—	—	—
Type II.							
6. Mt. Desert—Park, and 5 other cultures,	++	++	+	—	—	—	—
Type III.							
Manila—Flexner, and 5 other cultures,	++	++	++	++	++	++	+

Previous to immunization the serum agglutinated the bacilli of Type III. in 1 : 20 dilution, but none of the others even in 1:10. This is one of the few animals in which agglutinins for Type I. developed through the injections of bacilli of the other types.

TABLE IV.—*Showing how Type III. is unable to absorb the agglutinins produced through injections of Type II. Serum from rabbit inoculated with Mt. Vernon culture, Type II.*

	Serum before absorption.	Agglutinins exhausted with							
		Baltimore, Duval.					Mt. Vernon, c.c.[2]		
Type I.		1 : 20	1 : 50	1 : 100	1 : 200	1 : 400	1 : 20	1 : 50	1 : 100
Shiga, 5 other cultures,	1 : 10	—	—	—	—	—	—	—	—
Type II.									
Mt. Desert, 5 other cultures,	1 : 600	++	++	++	+ |	|	—	—	—
Type III.									
Manila, 5 other cultures,	1 : 100	—	—	—	—	—	++	|	—

Before injections this rabbit's serum agglutinated Types II. and III. in 1:20 dilutions.

The considerable amount of common agglutinins affecting Type II. and Type III. is seen to be absorbed by the bacilli of either type. The larger amount of specific agglutinin is left in the serum when any bacillus other than one of identical characteristics with the bacillus used in the immunization is employed.

TABLE V.—*Showing that horse injected with Shiga and Philippine types develop specific agglutinins for the bacilli belonging to these two types and common agglutinins for the varieties included under Type II.*

Cultures.	Serum after injections for several months.	Same serum after saturation with cultures of					
		Shiga Type.	Type III.	Type II.	Pyocyaneus.	Typhoid.	Colon.
Type I.							
Shiga, original, and 4 others,	+1500	—10	+400	+700	+1000	+300	+300
Type II.							
Park, original, and 4 others,	+600	—10	—10	—10	+600	+30	+50
Type II. (B.)							
Brooklyn,	+600	+20	+10	+50	+300	+100	+50
Type II. (C.)	+300	—10	—10	+50	+50	+10	+20
Type II. (D.)	+600	—20	—10	+50	+100	+30	+60
Type III.							
Flexner original and 4 others,	—1200	+400	—10	+500	+800	+300	+600

The manipulation necessary in making dilutions and filtering, as well as the effect of standing, cause a certain amount of destruction of agglutinins.

SUMMARY.—The great majority of the bacilli which have been isolated from cases of dysentery not due to amœbæ, and which must be considered as being exciting factors in that disease, are included in three distinct varieties or types. This at least is true for the many cultures which we have isolated or obtained from others.

The type most frequently found in severe epidemics is that of the first culture isolated by Shiga. Bacilli identical in biochemical and agglutinating characteristics with this bacillus have been isolated from cases of dysentery in many parts of the world. None of the bacilli belonging to this type produce indol, except, perhaps, in a trace, or ferment mannite, maltose, or saccharose. Animals injected with this type produce specific agglutinins for this type in abundance and only very little that combines with the others.

The second type ferments mannite with the production of acid, but does not split maltose or saccharose in peptone solution or agar.

It produces indol. Animals, after inoculations with it, develop immune bodies and agglutinins specific for the type. They also develop in considerable proportion immune bodies and agglutinins which have affinity for the bacilli of Type III. and to a slight extent for Type I.

The third type is nearest to the colon group, since it not only produces indol and actively ferments mannite, but also acts energetically upon pure maltose and feebly upon saccharose.

Animals injected with this type develop specific immune bodies and agglutinins, and also abundant immune bodies and agglutinins which have an affinity for the bacilli of Type II. and for many bacilli of the colon group. For Type I. these substances are but slightly developed.

These two mannite fermenting types are widely scattered over the world, and certainly cause characteristic cases and epidemics of dysentery, although on the average the disease caused by them is milder than when due to the Shiga bacillus. One or the other of these two types also appear at times in small numbers in mixed infections where dysenteric symptoms are almost or entirely absent.

Although the majority of bacilli obtained have had the characteristics of one of the above types, still bacilli have been found in isolated cases, which, although agreeing in biochemical characteristics with one of the three, nevertheless differed in producing different specific agglutinins. A few bacilli have also been met with which differ slightly in biochemical as well as agglutinating characteristics.

It seems, therefore, that these three types should be considered as the characteristic representatives of three groups.

In consideration of all the above facts, it seems to us incorrect to name the mannite-fermenting groups as pseudodysentery bacilli. If we call them all dysentery bacilli, we must classify them as dysentery bacilli of the Shiga group, of the group fermenting mannite, but not maltose, and of the one fermenting both mannite and maltose.

This manner of differentiating the groups would be very confusing, and it seems to us more convenient, and better, to restrict the name dysentery to bacilli having the characteristics of the bacillus isolated

by Shiga, and give the name paradysentery to the other two groups which approach more closely the colon group in that they produce indol and have a greater range of activity in fermenting carbohydrates.

An additional reason for the use of the prefix para, beyond that of convenience, is the less average severity of the disease due to these types, and the probability that there will be found, in occasional sporadic cases and epidemics of dysentery, bacilli which have a causal relation to dysentery and exhibit more pronounced characteristics of the colon group than any of the varieties so far isolated.

CHAPTER XX.

THE TYPHOID BACILLUS (BACILLUS TYPHOSUS).

THIS organism was first observed by Eberth, and independently by Koch, in 1880, in the spleen and diseased areas of the intestine in typhoid cadavers, but was not obtained in pure culture or its principal biological cultures described until the researches of Gaffky, in 1884. The methods of identification employed by Gaffky were found insufficient to separate the typhoid bacillus from other bacilli of the colon-typhoid group. Every known cultural characteristic of the typhoid bacillus was found to be duplicated in some member of this group, and it was only when a bacillus combined all the characteristics of a typical variety that it could be assumed that it was in all probability the typhoid bacillus. The absolute identification of the bacillus only became possible with the increase of our knowledge concerning the specific immune substances developed in the bodies of immunized animals. Its etiological relationship to typhoid fever has been particularly difficult of demonstration, for, although pathogenic for many animals when subcutaneously or intravenously inoculated, it has been impossible to produce infection in the natural way or produce gross lesions corresponding closely to those occurring generally in man. It has been recently shown, however, that animals under certain conditions, when their power of resistance has been reduced, may be rendered susceptible to infection, with the production of more or less characteristic lesions. These results, together with the specific reactions of the blood serum of typhoid patients, the constant presence of the bacillus typhosus in the intestines and some of the organs of the typhoid cadavers, the frequent isolation of this bacillus from the roseola, spleen, blood, and excretions of the sick during life, the absence of the bacilli in healthy persons, unless they have been directly exposed to or are convalescent from typhoid infection, all these have demonstrated scientifically that this bacillus is the chief etiological factor in the production of the great majority of cases designated as typhoid fever.

Morphology and Staining.—Typhoid bacilli are short, rather plump rods of about 1μ to 3μ in length by 0.5μ to 0.8μ in diameter, having rounded ends, and often growing into long threads. They are longer and somewhat more slender in form than most of the members of the colon group of bacilli (Figs. 88 and 89).

The typhoid bacilli *stain* with the ordinary aniline colors, but a little less readily than do most other bacteria. Like the bacilli of the colon and paratyphoid groups, they are decolorized by Gram's method.

Biology.—The typhoid bacillus is a motile, aërobic, facultative anaērobic, non-liquefying bacillus. It develops best at 37° C.; over 40° and below 30° growth is retarded; at 20° it is still moderate; below 10° it almost ceases. It grows slightly more abundantly in the presence of oxygen. It does not form spores.

Resistance.—When a number of typhoid bacilli are dried most of them die within a few hours and a few frequently remain alive for months, but sometimes all the bacilli die very quickly. In their

Fig. 88

Typhoid bacilli from nutrient agar. × 1100 diameters.

Fig. 89

Typhoid bacilli from nutrient gelatin. × 1100 diameters.

resistance to heat and cold they behave like the more resistant, non-spore-bearing bacilli.

Motility.—Typhoid bacilli, when living under favorable conditions, are very actively motile, the smaller ones having often an undulating motion, while the larger rods move about rapidly. In different cultures, however, the degree of motility varies.

Fig. 90

Flagella, heavily stained, attached to bacilli.

Fig. 91

Typhoid bacillus with stained flagella.

Flagella.—These are often numerous and spring from the sides as well as the ends of the bacilli, but many short rods have but a single terminal flagellum (Figs. 90 and 91).

Cultivation.—Its growth on most sugar-free culture media is similar to that of the bacillus coli communis, but it is somewhat slower and not quite so luxuriant.

Growth on Gelatin Plates (Fig. 92).—The colonies growing deep down in this plate medium have nothing in their appearance to distinguish them; they appear as finely granular round points with a sharp margin and a yellowish-brown color. The superficial colonies, however, particularly when young, are often quite characteristic; they are transparent, bluish-white in color, with an irregular outline, not unlike a grape-leaf in shape. Slightly magnified they appear homogeneous in structure, but marked by a delicate network of furrows. Surface colonies from some varieties of colon bacilli give a similar picture.

Fig. 92

2

1

A superficial colony (1) and a deep colony (2) of typhoid bacilli in gelatin. × 20 diameters.

In *stick cultures* in gelatin the growth is mostly on the surface, appearing as a thin, scalloped extension, which gradually reaches out to the sides of the tube. In the track of the needle there is but a limited growth, which may be granular or uniform in structure, and of a yellowish-brown color. There is no liquefaction.

Growth in Bouillon.—This medium is uniformly clouded by the typhoid bacillus, but the clouding is not so intense as by the colon bacillus. When the bouillon is somewhat alkaline a delicate film is sometimes formed on the surface after eighteen to twenty-four hours' growth.

Growth on Agar.—The streak cultures on agar are not distinctive; a transparent, grayish streak is formed.

Growth on Potato.—The growth on this medium was formerly of great importance in identification, but now other media, giving more specific characteristics, have been discovered. When characteristic the growth is almost invisible, but luxuriant, usually covering the surface of

the medium, and when scraped with the needle offers a certain resistance. In some cases, however, the growth is restricted to the immediate vicinity of the point of inoculation. Again, the growth may be quite heavy and colored yellowish-brown, and with a greenish halo, when it is very similar to the growth of the colon bacillus. These differences of growth on potato appear to be chiefly due to variations in the substance of the potato, especially in its reaction. For the characteristic growth the potato should be slightly acid. A new lot of potato should always be tested with a typical typhoid bacillus as a control.

Indol Reaction.—It does not produce indol. This test was proposed by Kitasato for differentiating the typhoid bacillus from other similar bacilli, such as those of the colon group, which, as a rule, give the indol reaction.

The typhoid bacillus, like the colon bacillus, produces alkaline substances from peptone.

Neutral Red.—In stick cultures in glucose agar the typhoid bacillus produces no change, while the colon bacillus decolorizes the medium and produces gas.

Effect of Inhibiting Substances in Culture Fluids.—The typhoid bacillus is inhibited by weaker solutions of formaldehyde, carbolic acid, and other disinfectants, than is the colon bacillus. Most typhoid-like bacilli resemble the typhoid bacillus in this respect.

Action on Different Sugars.—The determination of the action upon sugars of any bacillus belonging to the typhoid or colon group is one of the most important of all the cultural differential tests. It has been considered in detail in connection with the colon group.

Fermentation.—While the typhoid bacillus does not ferment glucose, galactose and levulose, it does produce acid from these substances. It evolves gas from none of the sugars.

Milk.—The typhoid bacillus does not cause coagulation when grown in milk. In litmus whey the neutral violet color becomes more red during the first forty-eight hours; the fluid, however, remains clear.

Production of Disease in Animals.—It is impossible experimentally to produce true typhoid fever in animals. Sickness or fatal results without the appearance of the typical pathological changes have regularly followed animal inoculations, but in most cases they could easily be traced to the toxæmia produced by the substances in the bodies of the bacilli injected. Typhoid bacilli, freshly obtained from typhoid cases and introduced subcutaneously in animals, rapidly die. In the peritoneal cavity they may increase, causing a fatal peritonitis with toxic poisoning. By accustoming bacilli to the animal body a certain degree of increased virulence for the animal can be obtained, so that smaller amounts of culture may prove fatal. Among the most successful efforts in this direction are the experiments of Cygnaeus and Seitz, who, by the inoculation of typhoid bacilli into dogs, rabbits, and mice, produced in the small intestines conditions that were histologically and to the naked eye analogous to those found in the human subject. Their results, however, were not constant.

Experiments indicate that the presence of other bacteria in the body, and of exposure to the effect of noxious gases in lowering the natural resistance of the individual, render him more susceptible to infection from typhoid fever.

Distribution of Bacilli in the Human Subject. Toxic Effects.—Typhoid fever belongs to that class of infectious diseases in which the specific bacilli may occur throughout the entire circulation, as in septicæmia, or remain localized in certain regions in the body. Wherever found in the tissues the typhoid bacilli are usually observed to be arranged in groups or foci; only occasionally are they found singly. These foci are formed, most probably, during life, as is proved by the degenerative changes often seen about them; but it is possible that the bacilli may also multiply somewhat after death.

The inflammatory changes in the lymphoid tissue and other cellular degenerations so often found in typhoid fever in the internal organs are due to the effects of the soluble toxic substances eliminated by the typhoid bacilli. The inflammation and ulceration of Peyer's patches are the central feature, these being more directly under the influence of the concentrated bacterial products. In typhoid fever necrosis of the tissues of the internal organs is of comparatively rare occurrence. Caseation of the mesenteric glands, which is commonly observed, is due possibly to mixed infection. There are, however, a number of cases now on record in which the typhoid bacillus has played the part of *pus producer*.

Unusual Location of Typhoid Lesions Occurring as Complications of Typhoid Fever.—Cases of sacculated and general peritonitis, subphrenic abscess, osteomyelitis, periostitis, and inflammatory procesess of other kinds have been reported as being due to the typhoid bacillus. Kruse also reports an abscess of the spleen which contained only bacillus typhosus, and typhoid abscess of the liver has been recorded by many. In certain cases of typhoid pneumonia, serous pleurisy, empyema, and inflammations of the brain and spinal cord or their membranes, typhoid bacilli exclusively have occurred. The inflammation produced may or may not be accompanied by the formation of pus.

Such cases, however, are of comparatively rare occurrence, because only exceptionally do the bacilli mass together in such numbers as to become pus producers. As a rule, when such complications occur in typhoid fever they are due to secondary or *mixed infection* with the staphylococcus, pneumococcus, streptococcus, pyocyaneus, and colon bacillus. Frequently these bacteria are found side by side with the typhoid bacilli; in such cases it is difficult to say which was the primary and which was the secondary infection.

The distribution of the typhoid bacilli in different parts of the body is explained by their passage through the circulation; and this is proved by the bacilli being found in the earlier days of the disease in the spleen constantly and frequently in the blood itself.

The typhoid bacillus can be transmitted also from the blood of the mother to the fœtus. In one case reported by Ernst a living child, four

days after birth, showed evidences of general typhoid infection—icterus and rose spots.

Not infrequently typhoid bacilli are found in the secretions. They are present in the urine in about 20 per cent. of the cases in the third and fourth weeks of typhoid fever. Slight pathological lesions in the kidneys almost always occur in typhoid fever, but severe lesions also sometimes occur. In some cases the urine is crowded with typhoid bacilli.

In cases of pneumonia due to the typhoid bacillus it is abundantly present in the sputa, and care should be taken to disinfect the expectoration of typhoid patients. In typhoid fever the bacilli are almost always present in the gall-bladder. The bacilli are usually eliminated by the feces, being derived from the ulcerated portions of the intestines; their growth within the intestinal contents is, with few exceptions, not extensive.

Not only do the very great majority of cases examined bacteriologically and pathologically, but the epidemiological history of the disease, proves that the chief mode of invasion of the typhoid bacillus is by way of the mouth and stomach. The infective material is discharged principally by means of the excretions and secretions of the sick—namely, by the feces, the urine, and occasionally by the sputum.

Occurrence in Healthy Persons.—In a few cases they have been obtained from the intestines of healthy persons. (Drigalski and Conradi, *Zeit. Hyg.*, vol. xxxix. p. 283.)

Duration of Life in Man.—The bacilli usually disappear from the body in the fourth or fifth week, but may remain for months or exceptionally years in the urine and in the gall-bladder. They have been found in collections of pus one year after recovery from typhoid fever.

Duration of Life Outside of the Body.—It is of importance to know for what length of time the typhoid bacillus is capable of living outside of the body; but, unfortunately, owing to the great difficulties in proving the presence of this organism in natural conditions, our knowledge on this point is still incomplete. In feces the length of life of the typhoid bacilli is very variable, depending on the composition of the feces and of the varieties of bacteria present; sometimes they live but a few hours, usually a day, exceptionally for very long periods. Thus, according to Levy and Kayser, in winter typhoid bacilli may remain alive in feces for five months. Foote says that they can be found in living oysters for a month at a time, but in numerous experiments we have not been able to find them after five days. Their life in privies and in water, however, is usually very much shorter. As a rule, they can be detected in river water no longer than seven days after introduction, and often not after forty-eight hours. The less contaminated the water, the longer the bacilli are apt to live. The life of the typhoid bacillus varies according to the abundance and varieties of the bacteria associated with it, and according to the presence or absence of such injurious influences as deleterious chemicals, high temperature, light, desiccation, etc., to which it is peculiarly sensitive. Good observers claim to have found

bacilli very similar to typhoid bacilli in the soil in a region where no typhoid fever was known to exist.

Communicability.—The bacilli may reach the mouth by means of infected fingers or articles of various kinds, or by the ingestion of infected food, milk, water, etc., or by more obscure ways, such as the eating of raw oysters and clams or the contamination of food by flies and other insects, or by inhalation through the mouth. Of the greatest importance, however, is the production of infection by contaminated drinking-water or milk. In a very large number of cases indirect proof of this mode of infection has been afforded by finding that the water had been contaminated with urine or feces from a case of typhoid. In a few instances the proof has been direct—namely, by finding typhoid bacilli in the water. Examples of infection from water and milk have frequently come under our direct observation. The following instances may be cited: A large force of workmen obtained their drinking-water from a well near where they were working. Typhoid fever broke out and continued to spread until the well was filled up. Investigation showed that some of the sick, in the early stages of their disease, repeatedly infected the soil surrounding the well with their urine and feces. Another example occurred in which typhoid fever broke out along the course of a creek after a spring freshet. It was found that, far up near the source of the creek, typhoid feces had been thrown on one of its banks and had then been washed into the stream.

In the late epidemic at Ithaca some 1500 cases developed among those using the infected water supply of the town. The students and towns-people not drinking the infected supply escaped.

An instance of milk infection secondary to water infection was in the case of a milk dealer whose son came home suffering from typhoid fever. The feces were thrown into a small stream which ran into a pond from which the milk cans were washed. A very alarming epidemic of typhoid developed, which was confined to the houses and asylums supplied with this milk. During the Spanish-American war not only water infection but food infection was noticed, as in the case of a regiment where certain companies were badly infected, while others nearly escaped. Each company had its separate kitchen and food supply, and much of the infection could be traced to the food, the contamination coming through the flies. Several epidemics have been traced to oysters.

Individual Susceptibility.—In this, as in all infectious diseases, *individual susceptibility* plays an important rọle in the production of infection. Without a suitable soil upon which to grow, the seed cannot thrive. There must in many be some disturbance of the digestion, excesses in drinking, etc., or a general weakening of the power of resistance of the individual, caused by bad food, exposure to heat, overexertion, etc., as with soldiers and prisoners, for example, to bring about the conditions suitable for the production of typhoid fever.

The supposition that the breathing of noxious gases predisposes to the disease, though possibly true to a certain extent, as some animal

experiments already referred to would seem to indicate, has not yet been conclusively proven; nor do Pettenkofer's investigations into the relation of the frequency of typhoid fever to the ground-water level satisfactorily explain the occurrence of the disease in most cases, whether sporadically or in epidemics.

Immunization.—After recovery from typhoid fever a considerable immunity is present which lasts for years. This is not absolute, as about 2 per cent. of those having typhoid fever have a second attack. This attack is usually a mild one. Specific *immunization* against experimental typhoid infection has been produced in animals by the usual method of injecting at first small quantities of the living or dead typhoid bacilli and gradually increasing the dose. The blood serum of animals thus immunized has been found to possess bactericidal and feeble antitoxic properties against the typhoid bacillus. These characteristics have also been observed in the blood serum of persons who are convalescent from typhoid fever. The attempt has been made to employ the typhoid serum for the cure of typhoid fever in man, but although a number of individual observers have reported good results with one or another of the sera most consider that little or no good is derived from the serum.

VACCINATION AGAINST TYPHOID.—The use of killed typhoid bacilli as vaccines has been advocated by Wright and tried upon some 8000 persons who expected to be subjected to danger of infection.

About 2 mg. of an agar tube culture which had been suspended in bouillon and heated was used at first, but now 0.3 to 0.1 c.c. of a bouillon culture according to the density of suspension is heated to 60° C. for five minutes. For a day or two the injection produces a slight fever and local pain, followed in a few days by the development of bactericidal substances in the blood, apparently sufficient in amount to give some immunity lasting for a year or more. A second injection adds to the degree of immunity. In 49,600 individuals under observation in India and Africa, 8600 were thus treated. The disease appeared in them to the extent of 2.25 per cent., with a case mortality of 12 per cent. In the 41,000 uninoculated there was a case percentage of 5.75 per cent., and a mortality of 26 per cent. The use of a protective serum, or, when this cannot be obtained, of dead cultures, would, therefore, seem to be advisable where great danger of typhoid infection exists.

Diagnosis by Means of the Widal or Serum Reaction.

The chief practical application of our knowledge of the specific substances developed in the blood of persons sick with typhoid fever has been as an aid to diagnosis.

In 1894–95 Pfeiffer showed that when cultures containing dead or living cholera spirilla or typhoid bacilli are injected subcutaneously into animals or man, specific protective substances are formed in the blood of the individuals thus treated. These substances confer a more or less complete immunity against the invasion of the living germs of the respective diseases. He also described the occurrence of a peculiar

phenomenon when some fresh culture of the typhoid bacillus on agar is added to a small quantity of serum from an animal immunized against typhoid bacilli and the mixture injected into the peritoneal cavity of a non-immunized guinea-pig. After this procedure, if from time to time minute drops of the liquid be withdrawn in a capillary tube and examined microscopically, it is found that the bacteria, previously motile and vigorous and which remain so in control animals inoculated without the specific serum, rapidly lose their motility and die. They are first immobilized, then they become somewhat swollen and agglomerated into balls or clumps, which gradually become paler and paler, until finally they are dissolved in the peritoneal fluid. This process usually takes place in about twenty minutes, provided a sufficient degree of immunity be present in the animals from which the serum was obtained. The animals injected with the mixture of the serum of immunized animals and typhoid cultures remain unaffected, while control animals treated with a fluid containing only the serum of non-immunized animals mixed with typhoid cultures die. Pfeiffer claimed that the reaction of the serum thus employed is so distinctly specific that it could serve for the differential diagnosis of the cholera vibrion or typhoid bacillus from other vibrions or allied bacilli, such as Finkler's and Prior's or those of the colon group.

In March, 1896, Pfeiffer and Kolle published an article entitled "The Differential Diagnosis of Typhoid Fever by Means of the Serum of Animals Immunized against Typhoid Infection," in which they claimed that by the presence or absence of this reaction in the serum of convalescents from suspected typhoid fever the nature of the disease could be determined. It was further found, if the serum of an animal thoroughly immunized to the typhoid bacillus was diluted with 40 parts of bouillon, and a similar dilution made of the serum of non-immunized animals, and both solutions were then inoculated with a culture of the typhoid bacillus and placed in the incubator at 37° C., that after the expiration of one hour macroscopic differences in the culture could be observed, which increased in distinctness for four hours and then gradually disappeared. The reaction occurring is described as follows: In the tubes in which the typhoid culture is mixed with typhoid serum the bacilli are agglomerated in fine, whitish flakes, which settle to the bottom of the tube, while the supernatant fluid is clear or only slightly cloudy. On the other hand, the tubes containing mixtures of bouillon with cholera or coli serum, or the serum of non-immunized animals inoculated with the typhoid bacilli, become and remain uniformly and intensely cloudy. These serum mixtures, examined microscopically in a hanging drop, show distinct differences. The typhoid serum mixture inoculated with the typhoid bacilli exhibits the organisms entirely motionless, lying clumped together in heaps; in the other mixtures the bacilli are actively motile.

Similar observations were made independently by Gruber and Durham, who maintained, however, that the reaction described by Pfeiffer was by no means specific, and that when the reaction is positive

the diagnosis still remains in doubt, for the reaction is *quantitative* only, and *not qualitative*. They concluded, nevertheless, that these investigations would render valuable assistance in the clinical diagnosis of cholera and typhoid fever.

Gruber-Widal Test.—The first application of the use of serum, however, for the early diagnosis of typhoid fever on a more extensive scale was made by Widal, and reported with great fulness and detail in a communication published in June, 1896. Widal confirmed the reaction as above described, proved that the agglutinative reaction usually occurred early, elaborated the test, and proposed a method by which it could be practically applied for diagnostic purposes. Since then the serum test for the diagnosis of typhoid fever has come into general use in bacteriological laboratories in all parts of the world, and though the extravagant expectations raised at the time when Widal first announced his method of applying this test have not been entirely fulfilled, it has, nevertheless, proved to be of great assistance in the diagnosis of obscure cases of the disease, and is now one of the recognized tests for the differentiation of the typhoid bacillus.

It should also be mentioned that to Wyatt Johnson, of Montreal, belongs the credit of having brought this test more conspicuously before the public, by introducing its use into municipal laboratories, suggesting that dried blood should be employed in place of blood serum (Widal having previously noticed that drying did not destroy the agglutinating properties of typhoid blood); and that in October, 1896, the serum test was regularly introduced in the New York Department of Health Laboratory for the routine examination of the blood serum of suspected cases of typhoid fever. Since then numerous health departments have followed the example set by those of Montreal and New York.

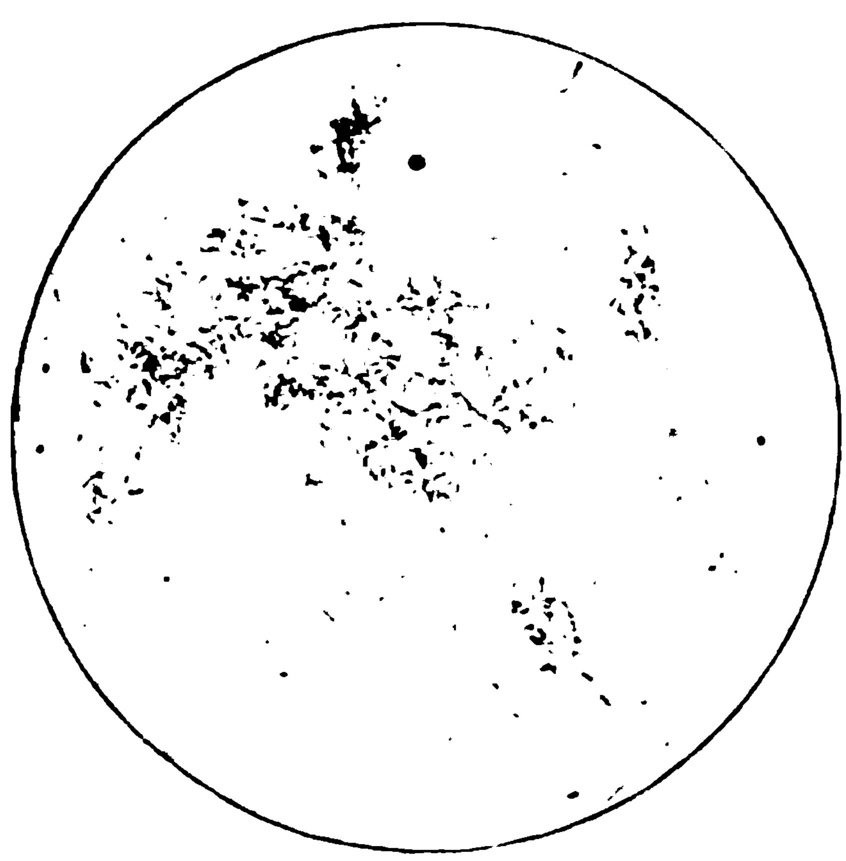
Use of Dried Blood. DIRECTIONS FOR PREPARING SPECIMENS OF BLOOD.—The skin covering the tip of the finger or the ear is thoroughly cleansed, and is then pricked with a needle deeply enough to cause several drops of blood to exude. Two fair-sized drops are then placed on a glass slide, one near either end, and allowed to dry. Glazed paper may also be employed, but it is not as good, for the blood soaks more or less into it, and later, when it is dissolved, some of the paper fibre is apt to be rubbed off with it. The slide is placed in a box for protection.

PREPARATION OF SPECIMEN OF BLOOD FOR EXAMINATION.—In preparing the specimens for examination the dried blood, if accuracy is desired, is first weighed and then brought into solution by adding to it and mixing it with nine times the quantity of water; then a minute drop of this decidedly reddish mixture is placed on a cover-glass, and to it is added a similar drop of an eighteen to twenty-four-hour-old bouillon culture of the typhoid bacillus, which, if it has a slight pellicle, should be well shaken. The drops, after being mixed, should have a faint reddish or pink tinge in this, the most highly concentrated serum dilution. Higher dilutions are prepared by adding sterile broth, water, or salt solution to the 1:5 blood mixture. The cover-glass with the mixture on the surface is inverted over a hollow slide (the edges about

the concavity having been carefully smeared with vaselin, so as to make a closed chamber), and the hanging drop then examined under the microscope by either daylight or artificial light, a high-power dry lens being used, or, somewhat less serviceably, a 1/12 oil-immersion lens. Ordinarily the dried blood is not weighed, but the measure of dilution is estimated by the color of the drop. To judge this the beginner must carefully make dilutions of fluid blood and notice the depth of color in 1:10 and 1:20 dilutions. Besides the faulty judgment of the dilution color by the examiner, the variation in depth of color of different specimens of blood makes the estimation of dilutions more or less inaccurate, but fortunately this does not greatly interfere with the value of the test.

The Reaction.—If the reaction takes place rapidly the first glance through the microscope reveals the reaction almost completed, most of the bacilli being in loose clumps and nearly or altogether motionless

FIG. 93



Gruber-Widal reaction. Bacilli gathered into one large and two small clumps, the few isolated bacteria being motionless or almost so.

(Fig. 93). Between the clumps are clear spaces containing few or no isolated bacilli. If the reaction is a little less complete a few bacilli may be found moving slowly between the clumps in an aimless way, while others attached to the clumps by one end are apparently trying to pull away, much as a fly caught on fly-paper struggles for freedom. If the agglutinating substances are present, but still less abundant, the reaction may be watched through the whole course of its development. Immediately after mixing the blood and culture together it will be noticed that the bacilli move more slowly than before the addition of serum. Some of these soon cease all progressive movement, and it will be seen that they are gathering together in small groups of two or more, the individual bacilli being still somewhat separated from each other. Gradually they close up the spaces between them, and clumps are

formed. According to the completeness of the reaction, either all of the bacilli may finally become clumped and immobilized or only a small portion of them, the rest remaining freely motile, and those clumped may appear to be struggling for freedom. With blood containing a large amount of agglutinating substances all the gradations in the intensity of the reaction may be observed, from those shown in a marked and immediate reaction to those appearing in a late and indefinite one, by simply varying the proportion of blood added to the culture fluid.

PSEUDOREACTIONS.—If too concentrated a solution of dried blood from a healthy person is employed a picture is often obtained which may be mistaken for a reaction. Dissolved blood always shows a varying amount of detritus, partly in the form of fibrinous clumps; and prolonged microscopic examination of the mixture of dissolved blood with a culture fluid shows that the bacilli, inhibited by substances in the blood, often become more or less entangled in these clumps, and in the course of one-half to one hour very few isolated motile bacteria are seen. The fibrinous clumps alone, especially if examined with a poor light by a beginner, may be easily mistaken for clumps of bacilli. Again, the bacilli may become fixed after remaining for one-half to two hours, by slight drying of the drop or the effect of substances on the cover-glass. The reaction in typhoid is chiefly due to specific substances, but clumping and inhibition of movement similar in character may be caused by other substances such as exist in normal horse and other serums. This is a very important fact to keep in mind. (For details of technique see pages 81–83.)

In pseudoreactions Wilson has noticed that many free bacilli are apt to be gathered at the margin of the hanging drop.

Use of Serum. MODE OF OBTAINING SERUM FOR EXAMINATION.—Fluid blood serum can easily be obtained in two ways: First, the serum may be obtained *directly from the blood,* thus: The tip of the finger or ear is pricked with a lancet-shaped needle, and the blood as it issues is allowed to fill by gravity a capillary tube having a central bulb. The ends of the tube are then sealed by heat or melted wax, or candle grease, and as the blood clots a few drops of serum separate. To obtain larger amounts of serum for a microscopic examination the blood is milked out from the puncture into a small homœopathic vial or test-tube. One cubic centimetre of blood can easily be collected in this way. The vial is then corked and placed on the ice to allow the serum to separate. As a rule one or two drops of serum are obtainable at the end of three or four hours. Second, the serum may be obtained *from blisters.* This gives more serum, but causes twelve hours' delay. The method is as follows: A section of cantharides plaster, the size of a 5-cent piece, is applied to the skin at some spot on the chest or abdomen. A blister forms in from six to eighteen hours. This should be protected from injury by a vaccine shield or bunion plaster. The serum from the blister is collected in a capillary tube, the ends of which are then sealed. Several drops of the serum can easily be obtained from a

blister so small that it is practically painless and harmless. The serum obtained is clear and admirably suited for the test.

ADVANTAGES AND DISADVANTAGES OF SERUM, DRIED BLOOD AND FLUID BLOOD FOR THE SERUM TEST.—The dried blood is easily and quickly obtained, and does not deteriorate or become contaminated by bacterial growth. It is readily transported, and seems to be of nearly equal strength with the serum in its agglutinating properties. It must in use, however, be diluted with at least five times its bulk of water, otherwise it is too viscid to be properly employed. The amount of dilution can only be determined roughly by the color of the resulting mixture, for it is impossible to estimate accurately the amount of dried blood from the size of the drop, and it is too much trouble to weigh it accurately. Serum, on the other hand, can be used in any dilution desired, varying from a mixture which contains equal parts of serum and broth culture to that containing 1 part of serum to 100 parts of culture or more, and this can be exactly measured by a graduated pipette, or, roughly, by a measured platinum loop. The disadvantages in the use of serum are entirely due to the slight difficulty in collecting and transporting it, and the delay in obtaining it when a blister is employed. If the serum is obtained from blood after clotting has occurred a greater quantity of blood must be drawn than is necessary when the dried-blood method is used; if it is obtained from a blister, a delay of six to eighteen hours is required. The transportation of the serum in capillary tubes presents no difficulties if tubes of sufficiently thick and tough glass are employed and placed in tiny wooden boxes. For scientific investigations and for accurate results, particularly in obscure cases, the use of fluid serum is to be preferred to dried blood. Practically, however, the results are nearly as good for diagnostic purposes from the dried blood as from the serum.

FLUID BLOOD.—When properly obtained this gives good results. The Thoma-Zeiss blood pipette is very useful. Lance finger-tip or ear and draw the blood into the pipette to the mark 0.5. Then *distilled water* is sucked up in sufficient amount to make the desired solution. One loop of this is added to one loop of bouillon culture.

THE CULTURE TO BE EMPLOYED.—It is important that the culture employed for serum-tests should be a suitable one, for although all cultures show the reaction, yet some respond much better and in higher dilutions than others. Cultures freshly obtained from typhoid cases are not as sensitive as those grown for some time on nutrient media. Decrease in virulence is apt to be accompanied by increase of capacity for agglutination. For the past seven years we have used a culture obtained from Pfeiffer. A broth culture of the typhoid bacillus developed at 25° to 35° C., not over twenty-four hours old, in which the bacilli are isolated and actively motile, has been found to give us the most satisfactory results. Cultures grown at temperatures over 38° C. are not apt to agglutinate so well as those grown at lower temperatures. Stock cultures of typhoid bacilli can be preserved on nutrient agar in sealed tubes and kept in the ice-box. These remain alive for months or

even years. From time to time one of these is taken out and used to start a fresh series of bouillon cultures.

Dilution of the Blood Serum to be Employed and Time Required for the Development of Reaction.—The serum test, as has been pointed out, is quantitative and not qualitative. By this it is not meant to assert that all the agglutinating substances produced in the blood of a patient suffering from typhoid infection are the same as those present in small amount in normal blood, or those produced in the blood of persons sick from other infections. It is true, however, that the apparent effect upon the bacilli is identical, the difference being that in typhoid fever, as a rule, substances which cause this reaction are usually far in excess of the amount which ever appears in non-typhoid blood, so that the reaction occurs after the addition to the culture of far smaller quantities of serum than in other diseases, or when the same dilution is used it occurs far more quickly and completely with the typhoid serum. The agglutinins which develop in animals after immunization with many bacilli comprise those which are specific and those which have affinity for widely differing varieties. (See chapter on Agglutinins.) It is most important to remember that it is purely a matter of experience to determine in any type of infection what degree of agglutinating strength in a serum is of diagnostic value.

The results obtained in the Health Department Laboratories, as well as elsewhere, have shown that in a certain proportion of cases not typhoid fever a slow reaction occurs in a 1:10 dilution of serum or blood; but very rarely does a complete reaction occur in this dilution within *fifteen minutes*. When dried blood is used the slight tendency of non-typhoid blood in 1:10 dilution to produce agglutination is increased by the presence of the fibrinous clumps, and perhaps by other substances derived from the disintegrated blood cells.

From many cases examined it has been found that in dilutions of 1:20 a quick reaction is almost never produced in any febrile disease other than that due to typhoid or paratyphoid bacillus infection, while in typhoid fever such a distinct reaction often occurs with dilutions of 1:100 or more. It is possible that some cases of paratyphoid infection give a prompt reaction in 1:20 dilutions, but if this is so, it is not a serious drawback.

The mode of procedure, therefore, as now employed is as follows: The test is first made with the typhoid bacillus in a 5 per cent. solution of serum or blood. In the case of serum, one part of a 1:10 dilution is added to one of the bouillon culture. With dried blood, a solution of the blood is first made, and the dilution guessed from the color. To obtain an idea of the dilution by the color, known amounts of blood are dried and then mixed with definite amounts of water; the colors resulting are fixed in the memory as guides for future tests. If there is no reaction—that is to say, if within five minutes no marked change is noted in the motility of the bacilli, and no clumping occurs—nothing

more is needed; the result is negative. If marked clumping and immobilization of the bacilli immediately begin and become complete within five minutes, this is termed *a marked immediate typhoid reaction*, and no further test is considered necessary, though it is always advisable to confirm the reaction with higher dilutions up to 1:50 or more, so as to measure the exact strength of the reaction. If in the 1:20 dilution a complete reaction takes place within thirty minutes, the blood is considered to have come from a case of typhoid infection, while if a less complete reaction occurs it is considered that only a probability of typhoid infection has been established. By many the time allowed for the development of the reaction with the high dilutions is from one to two hours, but to us thirty minutes with the comparatively low dilution of 1:20 seems safer and more convenient. Positive results obtained in this way may be considered conclusive, unless there be grounds for suspecting that the reaction may be due to a previous fairly recent attack. In our opinion the failure of the reaction in one examination by no means excludes the presence of typhoid infection. If the case clinically remains doubtful, the examination should be repeated within a few days.

USE OF DEAD CULTURES.—Properly killed typhoid bacilli respond well to the agglutination test. For the physician at his office the dead bacilli offer many advantages. The reaction is slower than with the living cultures and is observed either macroscopically or microscopically. A number of firms now supply outfits for the serum test. These outfits consist of a number of small tubes containing an emulsion of dead typhoid bacilli. Directions accompany the outfit.

PROPORTION OF CASES OF TYPHOID FEVER IN WHICH A DEFINITE REACTION OCCURS, AND THE TIME OF ITS APPEARANCE.—As the result of a large number of cases examined in the Health Department Laboratories, it was found that about 20 per cent. gave positive results in the first week, about 60 per cent. in the second week, about 80 per cent. in the third week, about 90 per cent. in the fourth week, and about 75 per cent. in the second month of the disease. In 88 per cent. of the cases in which repeated examinations were made (hospital cases) a definite typhoid reaction was present at some time during the illness.

PERSISTENCE OF THE REACTION.—In persons who have recovered from typhoid fever this peculiar property of the blood serum may persist for a number of months. Thus a definite typhoid reaction has been observed from three months to a year after convalescence, and a slight reaction, though much less than sufficient to establish a diagnosis of typhoid infection, from one to fifteen years after the disease.

REACTION WITH THE BLOOD SERUM OF HEALTHY PERSONS AND OF THOSE ILL WITH DISEASES OTHER THAN TYPHOID FEVER.—In the blood serum of over one hundred healthy persons examined in the Health Department Laboratories an immediate marked reaction has not been observed in a 1:10 dilution. In several hundred cases of diseases, eventually not believed by the physicians in charge to be typhoid

fever, only very rarely did the serum give a marked immediate reaction in a 1:10 dilution. In the light of past experience, I believe a typhoid or paratyphoid infection, though not a typical typhoid fever, to have existed in these cases. These results have been confirmed by others, the question of dilution having recently been made the subject of elaborate investigations, with the view of determining, if possible, at what dilution the typhoid serum would react while others would not. Thus, Schultz reports that among 100 cases of non-typhoid febrile diseases apparently positive results were obtained in 19 with dilutions of 1:5, in 11 of these with 1:10, in 7 with 1:15, in 3 with 1:20, and in 1 a very faint reaction with 1:25; whereas, in as many cases of true typhoid he never failed with dilutions of 1:50. In these experiments it must be noted, however, that the time limit was from one to two hours. A faint reaction with a 1:25 dilution with a time limit of two hours indicates less agglutinating substance than an immediate complete reaction with a 1:10 dilution.

From an experience with the practical application of the serum test for the diagnosis of typhoid fever extending over seven years, it may be said that this method of diagnosis is simple and easy of performance in the laboratory by an expert bacteriologist, but it is not to be recommended for routine employment by practising physicians as a clinical test unless they have had experience; that with the modifications as now employed, and due regard to the avoidance of all possible sources of error, it is as reliable a method as any other bacteriological test at present in use; and that as such the Gruber-Widal test is an indispensable, though not absolutely infallible, aid to the clinical diagnosis of irregular or slightly marked typhoid fever.

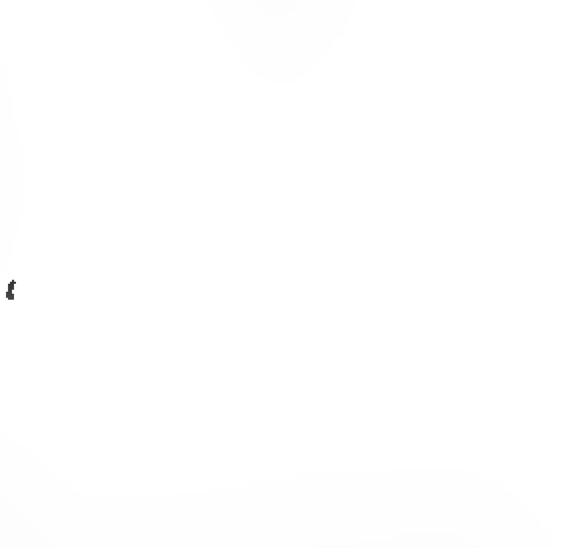
Isolation of Typhoid Bacilli from Suspected Feces, Urine, Blood, Water, etc.—In the bacteriological study of typhoid infection for diagnostic and other purposes, attempts have been made to isolate the specific bacilli from the blood, rose spots, sweat, urine, feces, and by spleen puncture. Although the results obtained by puncture of the spleen have been encouraging and have thrown light upon the distribution of the organism in the body during life, yet as a regular means of diagnosis it is to be discouraged, on account of the possible danger to the patient. The results of the examination of the blood and rose spots of typhoid patients have in the main proved unsatisfactory, though the investigations of some of the later observers have given a large percentage of positive results from the blood. The examination of the urine and feces of typhoid patients has more often given positive results than the blood, and these positive results have become more frequent and satisfactory as the methods for differentiating the bacillus typhosus have grown more exact and refined.

Several media recently devised for the isolation and identification of the typhoid bacillus are much better than any of those formerly used. These are the Hiss, Capaldi, Conradi, Drigalski, and Elsner media. In the hands of trained bacteriologists they give satisfactory results. The first three suffice for all ordinary purposes.

THE HISS MEDIA: THEIR COMPOSITION AND PREPARATION.[1]—Two media are used: one for the isolation of the typhoid bacillus by plate culture, and one for the differentiation of the typhoid bacillus from all other forms in pure culture in tubes.

The plating medium is composed of 10 grams of agar, 25 grams of gelatin, 5 grams of sodium chloride, 5 grams of Liebig's beef extract, 10 grams of glucose, and 1000 c.c. of water. When the agar is thoroughly melted the gelatin is added and completely dissolved by a few minutes' boiling. The medium is then titrated, to determine its reaction, phenolphthalein being used as the indicator. The requisite amount of normal hydrochloric acid or sodium hydrate solution is added to bring it to the desired reaction—*i. e.*, a reaction indicating 2 per cent. of normal acid. To clear the medium add one or two eggs, well beaten in 25 c.c. of water, boil for forty-five minutes, and filter through a thin filter of absorbent cotton. Add the glucose, after clearing. The reaction of the medium is most important; it should never contain less than 2 per cent. of normal acid.

FIG. 94



His' plate media: Small light colony (*t*) is composed of typhoid bacilli; large colony (*c*) of colon bacilli. (From Hiss.)

The tube medium contains agar, 5 grams; gelatin, 80 grams; sodium chloride, 5 grams; meat extract, 5 grams, and glucose, 10 grams to the litre of water, and reacts 1.5 per cent. acid by the indicator. The mode of preparation is the same as for the plate medium, care being taken always to add the gelatin after the agar is thoroughly melted, so as not to alter this ingredient by prolonged exposure to high temperature. The glucose is added after clearing. The medium must contain 1.5 per cent. of normal acid.

Growth of the Colonies.—The growth of the typhoid bacilli *in plates* made from the medium as above described gives rise to small colonies with irregular outgrowth and fringing threads (Fig. 94). The colon colonies, on the other hand, are much larger, and, as a rule, are darker in color and do not form threads. The growth of the typhoid bacilli *in tubes* produces uniform clouding at 37° C. within eighteen hours. The colon cultures do not give the uniform clouding, and present several appearances, probably dependent upon differences in the degree of their motility and gas-producing properties in media. Some of the varieties of the colon bacillus grow only locally where they were inoculated by the platinum needle. Others grow diffusely through the medium, but owing to the production of gas and the passage of

[1] This description is taken from an article by Dr. Philip Hanson Hiss, Jr., "On a Method of Isolating and Identifying Bacillus Typhosus and Members of the Colon Group in Semisolid Culture Media," published in the Journal of Experimental Medicine, 1897, vol. ii., No. 6.

gas-bubbles through the medium, clear streaks ramify through the otherwise diffusely cloudy tube contents. This characteristic appearance is not produced when the medium is incorrect in reaction or in consistency. With untried media it is always well to insert a platinum wire into the tube contents and stir them about; if any gas is liberated the culture is not one of the typhoid bacillus and the medium is not correct.

Method of Making the Test.—The usual method of making the test is to take enough of the specimen of feces or urine—*i. e.*, from one to several loops—and transfer it to a tube containing broth. From this emulsion in broth five or six plates are generally made by transferring one to five loops of the emulsion to tubes containing the melted plate medium, and then pouring the contents of these tubes into Petri dishes. These dishes are placed in the incubator at 37° C. and allowed to remain for eighteen to twenty-four hours, when they may be examined. If typical thread-forming colonies are found the tube medium is inoculated from them, and the growth in the tubes allowed to develop for about eighteen hours at 37° C. If these tubes then present the characteristic clouding, experience indicates that the diagnosis of typhoid may be safely made, for the typhoid bacillus alone, of all the organisms investigated, has displayed the power of giving rise both to the thread-forming colonies in the plating medium and the uniform clouding in the tube medium when exposed to a temperature of 37° C. The organisms isolated in this manner have been subjected to the usual tests for the recognition of the bacillus typhosus, and have always corresponded in all their reactions to those given by the typical typhoid bacillus.

THE CAPALDI PLATE MEDIUM.—In his original paper, Capaldi gives the following recipe:

Aqua dest.	1000
Gelatin	20
Mannite (grape-sugar)	10
Sodium chloride	5
Potassium chloride	5

Boil, filter, add 2 per cent. agar and 10 c.c. of normal sodic hydrate solution; boil, filter, and sterilize.

In making up the medium for work the only variation was that in the original recipe the agar was added when the gelatin was put in, and the gelatin was added after the first filtration.

The Capaldi medium is usually employed for surface cultures, but can be inoculated while melted in the tubes. Plates may be made beforehand, so that they are ready for use when the specimen comes in. As these plates are to be kept at 37° C., the difficulties in regard to temperature are avoided; but, unlike the Elsner plates, other organisms besides the colon and typhoid develop and may cause some confusion. In making the plates one or two are inoculated by gently carrying across their surface a platinum loop of feces or urine. Others are

then inoculated with a loop of urine or much diluted feces. In this way we are apt to have some plates with just the right amount of colonies.

Appearance of the Colonies.—Capaldi thus describes the differentiation: Typhoid—Small, glistening, transparent, almost colorless colonies (by reflected light, blue). Colon—Large, milky colonies (reflected light, brown).

In using the medium it was found that even in a pure plate of typhoid the colonies vary much in size and appearance, while different typhoids show individual differences in growth. In general, a medium-sized, *gray-white* colony, with a few refractive granules, is the typhoid. However, it is often transparent, without the refractive granules; sometimes with a nuclear centre, and sometimes of equal consistency throughout. Streptococci simulate typhoid, but a high-power lens will show the coccus.

Colon colonies are usually much larger than the typhoid—a decided brown color, very large, refractive granules, and in general quite different in appearance (Fig. 95).

FIG. 95

Colonies of colon bacilli on Capaldi medium slightly magnified. Typhoid colonies of same size usually have no dark granules.

The best way to work with the Capaldi medium is to make several plates with different typhoid cultures, observe carefully all the variations in the colonies, and bear these in mind when working with the mixed plates. After these precautions have been taken the medium will be found very satisfactory. The colonies, as a rule, appear characteristically in twelve to eighteen hours, and thus give a quick method of diagnosis.

We found that the two media (Capaldi and Hiss) work excellently together, as one is an aid to the other. When many colonies of the typhoid bacilli were present the points of differentiation were usually

easily seen upon both media, and the two together made diagnosis almost certain. The bacilli from the suspected typhoid colonies can be quickly tested, sufficiently for practical purposes, on the Hiss tube medium, and by the reaction between the bacilli and the serum from an immunized horse.

TYPHOID MEDIUM OF VON DRIGALSKI AND CONRADI.—These authors modified lactose litmus agar by adding to it nutrose and crystal violet and by using 3 per cent. of agar instead of 2 per cent. The crystal violet strongly inhibits the growth of many other bacteria, especially cocci, which would also color the medium red; the 3 per cent. agar makes the diffusion of the acid which is formed more difficult.

Three pounds of chopped beef are allowed to stand twenty-four hours with 2 litres of water. The meat infusion is boiled one hour and filtered. 20 grams Witte's peptone, 20 grams nutrose, and 10 grams of salt are then added, and the mixture boiled another hour. After filtration and the addition of 60 grams agar the mixture is boiled for three hours, alkalized and filtered. In the mean time 300 c.c. litmus solution (Kahlbaum) are boiled for fifteen minutes with 30 grams lactose. Both solutions are then mixed and the mixture, which is now red, faintly alkalized with 10 per cent. soda solution. To this feebly alkaline mixture 4 c.c. hot sterile 10 per cent. soda solution are added and 20 c.c. of a sterile solution (0.1:100) of crystal violet Höchst B.

Plates are made of this in the usual way. The material to be examined (stools first diluted with ten volumes of 0.8 per cent. salt solution) is spread directly on the surface of the plates, and these then allowed to stand slightly open for about half an hour in order that they may dry somewhat. They are then placed inverted into the incubator for from sixteen to twenty-four hours. Typhoid colonies are small (1 to 3 mm.), transparent, and blue; colon colonies are red, coarser, less transparent, and larger. The suspected colonies can at once be tested for agglutination with a high grade typhoid serum.

In general this method has withstood critical tests and it is nowadays regarded as one of the very best.

As to the comparative merits of the four media, it is probably safe to say that any one of them will, in the hands of one accustomed to them, reveal the typhoid bacilli, except perhaps when they exist in only the most minute numbers. The Elsner method has the objection that it is very difficult to work with in hot weather. The Hiss plate medium has the objection that it is a difficult medium to prepare. If the acidity is not just right the thread outgrowths do not appear. Indeed, the only sure way is to test a new batch of medium with a pure culture and alter the reaction until the culture grows correctly. A very few varieties of the typhoid bacillus do not produce typical thread outgrowths from the colonies. In the Drigalski medium the typhoid colonies are easily separated from those of the colon bacilli, but there are other intestinal bacteria which grow like them.

The Capaldi medium has the objection that some of the typhoid and some of the colon colonies frequently look much alike. If one, however, will always pick out the colonies which look most like the typhoid, it will usually turn out that typhoid bacilli have been obtained if any were present. Personally, for general use, I prefer the Capaldi or Drigalski medium for the plate cultures and the Hiss tube medium for identifying the bacilli obtained. Through these media and specific agglutinating serum we are now in a position to obtain and identify typhoid bacilli from feces, urine, etc., within forty-eight hours.

Typhoid Bacilli in Feces.—Recently numerous investigations have been carried out to discover how frequently and at what period in typhoid fever bacilli were present in the feces and urine. Hiss some time ago examined the feces in 43 consecutive cases, 37 of which were in the febrile stage and 6 convalescent. In a number of instances only one stool was examined, but even under these adverse conditions the average of positive results in the febrile stage was 66.6 per cent. Out of 26 cases of typhoid fever examined in hospitals, 21 were in the febrile stage and 5 convalescent. In the febrile cases in 17 the presence of typhoid bacilli, often in great numbers, was demonstrated. Thus in these carefully followed cases the statistics show over 80 per cent. of the febrile cases positive. The bacilli were isolated from these cases as early as the sixth day, and as late as the thirtieth day, and in a case of relapse on the forty-seventh day of the disease. The convalescent cases gave uniformly negative results, the earliest examination having been made on the third day after the disappearance of the fever. The bacilli seemed to be more numerous in the stools from about the tenth or twelfth day on. These observations, with regard to the appearance of the bacilli in the stools during the febrile stage and their usually quick disappearance after the defervescence, have been confirmed by others. The bacilli were isolated in several cases in which no Widal reaction was demonstrated. Between the seventh and twenty-first days of the disease, experience seems to indicate that the bacilli may be obtained from about 25 per cent. of all cases on the first examination and from about 75 per cent. after repeated examinations. In some samples of feces typhoid bacilli die out within twenty-four hours; in others they remain alive for days or even weeks. This seems to depend on the bacteria present in the feces and upon its chemical character. Probably the presence of typhoid bacilli in some stools and their absence in others must be explained largely by the characteristics of the intestinal contents. The short life of the typhoid bacillus in many specimens of feces suggests that stools be examined as quickly as possible. In fact, unless the physician wishes to take the trouble to have the sample of feces sent immediately to the laboratory, it is hardly worth while for the bacteriologist to take the trouble to make the test.

Typhoid Bacilli in the Urine.—Of even more interest than the presence of the bacilli in feces is their frequent occurrence in great numbers in the urine. The results of the examinations of others as

well as our own indicate that the typhoid bacilli are not apt to be found in the urine until the beginning of the third week of the fever, and may not appear until much later. From this on to convalescence they appear in about 25 per cent. of the cases, usually in pure culture and in enormous numbers. Of 9 positive cases examined by Richardson[1] 2 died and 7 were discharged. At the time of their discharge their urine was loaded with typhoid bacilli. We have observed similar cases. In one the bacilli persisted for five weeks. Undoubtedly in exceptional cases they persist for years. When we think of the chances such cases have to spread infection as they pass from place to place, we begin to realize how epidemics can start without apparent cause. The more we investigate the persistence of bacteria in convalescent cases of disease, the more difficult the prevention of their dissemination is seen to be. The disinfection of the urine should always be looked after in typhoid fever, and convalescents should not be allowed to go to places where contamination of the water supply is possible, without at least warning them of the necessity of great care in disinfecting their urine and feces for some weeks. Richardson made the interesting discovery that after washing out the bladder with a very weak solution of bichloride of mercury the typhoid bacilli no longer appeared in the urine.

Paratyphoid.—A few of the cases of "typhoid" heretofore described as giving no Gruber-Widal reaction were undoubtedly due to the *paratyphoid bacilli*. As has been already stated, this is the name by which we now, in conformity with Schottmüller, designate a bacillus which stands about midway between B. typhosus and B. coli. It has been found necessary to distinguish two varieties, *type A* and *type B*, which differ also in their agglutinating property. It remains to be seen whether we shall have to differentiate any additional types. There are no certain distinguishing features to separate the clinical pictures of abdominal typhoid and paratyphoid. Many cases of paratyphoid present all the classical symptoms of typhoid. According to Conradi, von Drigalski, and Jürgens the fever curve of paratyphoid is characterized by a fairly sudden rise, an irregular course of the temperature with almost always an absence of the continua. Besides this, the disease has a better prognosis and a slow convalescence. According to other authors, enlargement of the spleen is quite often absent (de Feyfer and Kayser missed it in 42 per cent. of the cases), whereas an involvement of the upper portions of the intestinal tract (gastric fever!) is more common. Further than this, it is unwise to lay much stress on peculiarities in the course of the disease, for we know that true typhoid runs a variable course. We have only to think of the vast difference between a mild or abortive typhoid and a fully developed or, better still, a complicated case. It will almost always be impossible to separate a case of true typhoid from a paratyphoid by the symptoms alone. At the most, during an epidemic the general course of the disease, when it agrees

[1] Journal of Experimental Medicine, May, 1898.

with the above points, may cause one to suspect paratyphoid. Schottmüller and Kurth, from a total of 180 cases which had been looked upon as typhoid, were able in 12 cases to isolate a paratyphoid bacillus.

Hünermann observed a whole epidemic in which typhoid-like bacteria, which he regarded as the cause of the disease, were found in the blood. The Gruber-Widal reaction 1:100 was positive in only 42 per cent.; the newly found bacillus was always agglutinated. Similar reports concerning an epidemic of 14 cases in Holland are made by de Feyfer and Kayser; and Sion and Negel report one from Roumania. Formerly none of these cases would have been differentiated from true typhoid.

Detection of Typhoid Bacilli in Water.—There is absolutely no doubt that the contamination of streams and reservoirs is a frequent cause of the outbreak of epidemics of typhoid fever, but the actual finding and isolation of the bacilli is a very rare occurrence. This is often due to the fact that the contamination has passed away before the bacteriological examination is undertaken, and also to the great difficulties met with in detecting a few typhoid bacilli when they are associated with large numbers of other bacteria. The greater the amount of contamination entering the water, and the shorter the time which elapses between this and the drinking of the water, the greater is the danger.

Differential Diagnosis.—The typhoid bacillus and the bacilli of the colon group resemble each other in many respects. It is necessary to remember that there are many varieties of bacilli differing in both cultural and agglutination reactions which are grouped under the general name of the colon bacillus. By comparing what has been said of the bacillus coli and the bacillus typhosus it will be seen that while certain varieties of each simulate each other in many respects, the characteristic varieties of each still possess individual characteristics by which they may be readily differentiated:

1. The motility of the B. coli is, as a rule, much less marked than that of the B. typhosus. The colon bacillus is also shorter, thicker, and has fewer flagella.

2. In gelatin the colonies of the colon bacillus develop more rapidly and luxuriantly than those of the typhoid bacillus.

3. On potato the growth of the colon bacillus is usually rapid, luxuriant, and visible, though not invariably so; while that of the typhoid bacillus is ordinarily invisible.

4. The characteristic colon bacillus coagulates milk in from thirty-six to forty-eight hours in the incubator, with acid reaction. The typhoid bacillus does not cause coagulation.

5. The colon bacillus is conspicuous for its power of causing fermentation, with the production of gas in media containing glucose. The typhoid bacillus never does this.

6. In nutrient agar or gelatin containing lactose and litmus tincture, and of a slightly alkaline reaction, the color of the colonies of the colon

bacillus is pink, and the surrounding medium becomes red; while the colonies of the typhoid bacillus are blue, and there is little or no reddening of the surrounding medium. The same points hold true on the Drigalski-Conradi medium.

7. The colon bacillus possesses the property of producing indol in cultures of bouillon or peptone; the characteristic typhoid bacillus does not produce indol in these solutions.

8. The colon bacillus rarely produces thread outgrowths in properly prepared Hiss plate medium. The typhoid bacillus produces thread outgrowths and smaller colonies in this medium. In the Hiss tube medium the colon bacillus produces either a growth limited to the area inoculated or a diffuse growth streaked with clear lines and spaces. The typhoid bacillus produces a diffuse growth, evenly clouding the entire medium.

9. On the Capaldi medium the colon colonies are more granular and darker than those of the typhoid bacilli.

10. Finally, on testing the bacilli in the hanging drop with the serum of animals immunized to the typhoid bacillus, the typhoid bacilli become agglutinated in high dilutions of the serum, while the colon bacilli do not.

None of these tests alone can be depended upon for making a differential diagnosis of the colon bacillus from the typhoid bacillus or other similar bacilli.

Unfortunately, also, in most of these characteristics certain degrees of variation may often be observed and these may lead to confusion. For instance, the morphology may vary considerably, at times even when grown on the same culture media, and the motility is not always equally pronounced; the flagella may vary; the rapidity of growth may differ, especially between freshly made and old cultures; the grape-leaf appearance of the surface colonies on gelatin, which is usually characteristic, may vary with the composition of the gelatin, at times no typical colonies at all being presented; the threads in the Hiss media may be lacking; in rare instances the typhoid bacillus produces indol; the growth on potato is not to be depended on, often being visible and not characteristic; the virulence of both the bacilli is so little characteristic that it can hardly be used for diagnostic purposes; and finally, the serum test is not to be depended on unless the agglutinins in the serum have been properly tested, for there is abundant agglutinin for some of the colon bacilli in the serum of many untreated animals. This is less true of rabbits than of horses and of young than older animals.

In spite, however, of these difficulties it is very easy to sufficiently identify the typhoid bacillus for all practical purposes. A bacillus which grows typically in the Hiss tube media, and shows agglutination with a high dilution of the serum of an animal immunized to the typhoid bacillus, is in all probability the typhoid bacillus. If this bacillus absorbs the specific typhoid agglutinins it is undoubtedly the typhoid bacillus. The same could probably be said of a bacillus which grew

characteristically in glucose bouillon and nutrient gelatin, besides showing the specific serum reaction. Probably not one time in ten thousand would such bacilli prove on further investigation not to be typhoid bacilli. A still further test is to inoculate animals with several doses of the dead bacilli whose identification is sought, and note whether there is produced a serum which specifically agglutinates undoubted typhoid bacilli.

CHAPTER XXI.

THE BACILLUS OF TUBERCULOSIS.

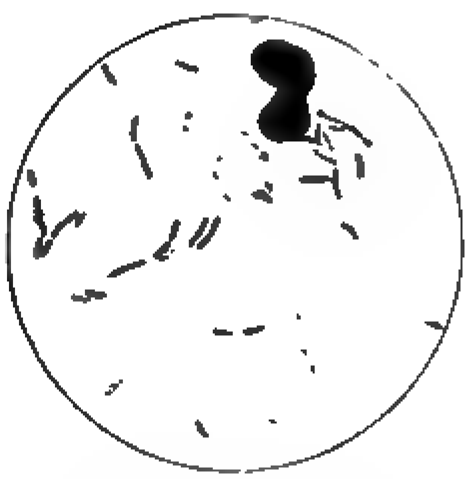
A KNOWLEDGE of phthisis was certainly present among men at the time from which our earliest medical descriptions come. For over two thousand years many of the clearest-thinking physicians have considered it a communicable disease; but it is only within comparatively recent times that the infectiousness of tuberculosis has become an established fact in scientific medicine. Villemin, in 1865, by infecting a series of animals through inoculations with tuberculous tissue, showed that tuberculosis might be induced, and that such tissue carried the exciting agent of the disease. Baumgarten demonstrated, early in 1882, bacilli in tissue sections which are now known to have been the tubercle bacilli. But these investigations and those of others at the same time, though paving the way to a better knowledge of the disease, proved to be unsatisfactory and incomplete. The announcement of the discovery of the tubercle bacillus was made by Koch in March, 1882. Along with the announcement satisfactory experimental evidence was presented as to its etiological relation to tuberculosis in man and in susceptible animals, and its principal biological characters were given. An innumerable number of investigators now followed Koch into this field, but their observations served only to confirm his discovery.

Distribution of Bacilli.—They are found in the sputum of persons and animals suffering from pulmonary or laryngeal tuberculosis, either free or in the interior of pus cells; in miliary tubercles and fresh caseous masses in the lungs and elsewhere; in recent tuberculous cavities in the lungs; in tuberculous glands, joints, bones, mucous membranes, and skin affections.

Morphology.—The tubercle bacilli are slender, non-motile rods of about 0.3μ in diameter by 1.5 to 4μ in length. (Plate I., Figs. 1, 2, and 3.) Commonly they occur singly or in pairs, and are then usually slightly curved; frequently they are observed in smaller or larger bunches. Under exceptional conditions branching and club-shaped forms are observed. Injected into the brain, kidney, and other tissues in rabbits a growth frequently occurs in which forms similar to actinomyces develop. The tubercle bacillus clearly belongs among the higher forms of bacteria and is closely allied to actinomycosis. The same is true for some of the timothy and other acid-fast bacilli. In stained preparations there are often seen unstained portions. From two to six of these vacuoles may sometimes be noticed in a single rod. In old cultures irregular forms may develop, the rods being occasionally swollen at one end or presenting lateral projections. Here also spherical granules

PLATE I.

FIG 1



Tubercle bacilli, in red.
Streptobacilli, in blue.

× 1100 diameters.

FIG. 2.

Tubercle bacilli, in red.
Tissue, in blue.

× 1100 diameters

FIG. 3.

Leprosy bacilli in nasal secretion of person suffering from nasal lesions. (Hansen.)

× 500 diameters.

FIG 4.

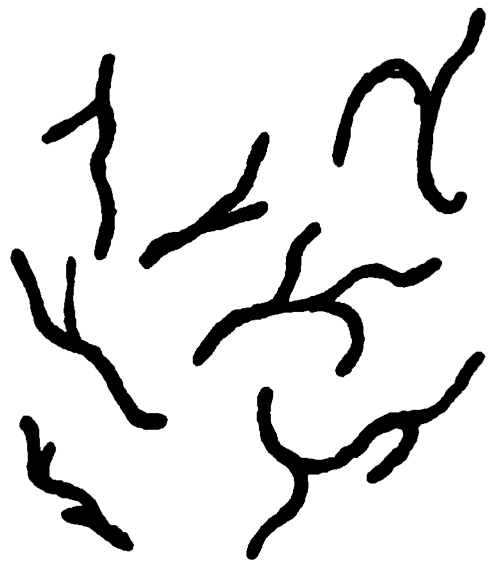
Short smegma bacilli.
Bacilli in specimen are red, rest of material in blue.

× 1100 diameters.

appear which stain with more difficulty than the rest of the bacillus and also retain the stain with greater tenacity. The bacilli, however, containing these bodies are not appreciably more resistant than those not having them; although, therefore, these bodies have some of the characteristics of a spore, they lack the quality of resistance to deleterious influences and cannot be considered true spores.

The bacilli have a thin capsule, shown in one way by the fact that they appear thicker when stained with fuchsin than with methylene blue. The capsule is believed to contain the greater portion of the wax-like substance peculiar to the bacillus.

FIG. 96



Branched forms. (From C. F. Craig.)

Staining Peculiarities.—These are very important, for by them its differentiation and recognition in microscopic preparations of sputum, etc., are rendered possible. Owing to the waxy substance in its envelope it does not readily take up the ordinary aniline colors, but when once stained it is very difficult to decolorize, even by the use of strong acids. The more recently formed bacilli are much more easily stained and decolorized than the older forms. Ehrlich devised a method of staining which proved to be satisfactory—viz., the use of a solution of an aniline color—fuchsin or methyl violet—in a saturated aqueous solution of aniline oil and decolorization of other bacteria with a solution of a mineral acid, to be followed by a contrast stain, such as methylene blue. (Plate, I., Figs. 1 and 2.) Various modifications of Ehrlich's method are now commonly used. The tubercle bacilli can be demonstrated also by Gram's method of staining.

Biology.—The bacillus tuberculosis is a *parasitic, aërobic, non-motile* bacillus, and grows only at a temperature of about 37° C., limits 30° to 42° C. It does not form true spores.

RESISTANCE.—The bacilli, possibly on account of the nature of their capsule, have a somewhat greater resisting power than most other pathogenic bacteria, since frequently the bacilli resist desiccation at the ordinary temperatures for months; most bacilli die, however, soon after drying. Upon serum cultures the bacilli seldom live longer than six to eight months. They frequently retain their vitality for several weeks in putrefying material, such as sputum. Cold has little effect upon them. When dry the more resistant organisms stand dry heat at 100° C. for hours; but when moist, as in milk, they are quickly killed—viz., at 55° C. in four hours, at 60° C. in thirty minutes, at 65° C. in fifteen minutes, at 70° C. in ten minutes, at 80° C. in five minutes, and at 95° C. in one minute. One reason why in some experiments they appear to withstand high temperatures is, as pointed out by Theobald Smith, that when heated in a test-tube in the usual way the cream which rises on heating is exposed on its surface to a lower temperature than the rest of the milk, and as this contains the

greatest percentage of the bacteria some of them are exposed to less heat than those in the rest of the fluid receive.

The resisting power of this bacillus to chemical disinfectants, drying, and light is considerable, but not as great as it is apt to appear, for, as in sputum, the bacillus is usually protected by mucus or cell protoplasm from penetration by the germicidal agent. It is not always destroyed by the gastric juice in the stomach, as is shown by successful infection experiments in susceptible animals by feeding them with tubercle bacilli. They are destroyed in sputum in six hours or less by the addition of an equal quantity of a 5 per cent. solution of carbolic acid. Bichloride of mercury is less suitable for the disinfection of sputum. Iodoform has no effect upon cultures until 5 per cent. is added. The fumes from four pounds of burning sulphur to each 1000 cubic feet of air space will kill tubercle bacilli in eight hours when fully exposed to the action of the gas, providing that they are moist, or that abundant moisture is present in the air.

Formaldehyde gas is quicker in its action, but not much more efficient. Ten ounces of formalin should be employed for each 1000 cubic feet of air space.

The tubercle bacillus in sputum when exposed to direct sunlight is killed in from a few minutes to several hours, according to the thickness of the layer and the season of the year; it is also usually destroyed by diffuse daylight in from five to ten days when placed near a window. Protected in cloth the bacilli survive exposure to light for longer periods. This fact is worthy of note, as it has an important hygienic bearing. Thus, tuberculous sputum expectorated upon sidewalks, etc., being often exposed to the action of direct sunlight, will in many cases, especially in summer, be disinfected by the time it is in a condition to be carried into the air as dust. For this and other more important hygienic reasons, consumptive patients should occupy light, sunny rooms and live as much as possible in the open air.

Dried sputum in places protected from abundant light has occasionally been found to contain virulent tubercle bacilli for as long as ten months. For a year at least it should be considered dangerous. The Roentgen rays have a deleterious effect on tubercle bacilli in cultures, but practically none upon those in tissues.

The tubercle bacillus is a strict parasite—that is to say, its biological characters are such that it could scarcely find natural conditions outside of the bodies of living animals favorable for its multiplication. Under exceptional conditions, such as in freshly expectorated sputum, tubercle bacilli may increase for a limited time.

Cultivation of the Tubercle Bacillus.—On account of their slow growth and the special conditions which they require, tubercle bacilli cannot be grown in pure culture by the usual plate method on the ordinary culture media. Koch first succeeded in cultivating and isolating this bacillus on coagulated blood serum, which he inoculated by carefully rubbing the surface with sections of tuberculous tissue and then leav ng the culture, protected from evaporation, for several weeks

in the incubator. Cultures are more readily obtained from human than from bovine bacilli.

Growth on Coagulated Blood Serum or Egg.—On these media, one of wh ch is regularly used to obtain the first culture, the growth first becomes visible at the end of ten to twenty-one days at 37° C., and at the end of three to four weeks a distinct and characteristic development has occurred Small, grayish-white points and scales first appear on the surface of the medium. As development progresses there is formed an irregular, membranous-looking layer. When a tiny piece of this is removed, placed on a cover-glass without rubbing, stained, and then observed under the microscope, the surface growth presents a characteristic appearance, the bacilli being arranged in parallel rows of variously curved figures.

Owing to the greater facility of preparing and sterilizing *glycerin agar*, and the more rapid and abundant growth of the bacilli, which have become accustomed to growth outside the body on this medium, it is now usually employed in preference to blood serum for continuing to produce later cultures. The development at the end of fourteen to twenty-one days is more abundant than upon blood serum after several weeks. When numerous bacilli have been distributed over the surface of the culture medium, a rather uniform, thick, white layer, which subsequently acquires a slightly yellowish tint, is developed; when the bacilli sown are few in number, or are associated in scattered groups, separate colonies are developed, which acquire considerable thickness and have more or less irregu'ar outlines.

Growth on Nutrient Veal or Beef Broth Containing 5 per cent. of Glycerin.—This is of importance, because in this way tuber-

Fig. 97

Growth of tubercle bacilli upon glycerin bouillon. (Kolle and Wassermann.)

culin is produced. On these media the tubercle bacillus also grows readily if a very fresh thin film of growth from the glycerin agar is floated on the surface. The latter of these media is used for the development of tuberculin. The small piece of pellicle removed from the previous culture cont'nues to enlarge while it floats on the surface of the liquid, and in the course of three to six weeks covers it wholly as

a single film, which on agitation is easily broken up and then settles on the bottom of the flask, where it ceases to develop further. The liquid remains clear. A practical point of importance, if a quick growth is desired, is to remove for the new cultures a portion of the pellicle of a growing bouillon culture, which is very thin and actively increasing.

Obtaining of Pure Cultures of the Tubercle Bacillus from Sputum, Infected Tissue and Other Materials.—On account of the time required and the difficulties to be overcome this is never desirable except when careful investigations of importance are to be undertaken. The chief point of present interest is the dissemination of bovine bacilli in man. The discovery by Theobald Smith of the greater acid production of the human type of tubercle bacilli in glycerin bouillon over the bovine bacilli has made it a matter of added importance to test as many bacilli as possible for their biochemical characteristics. *Pure cultures* can be obtained directly from tuberculous material when mixed infection is not present, and a suitable dog serum or egg culture medium is at hand; but as it is so difficult to get material free from other bacteria which grow much more rapidly and take possession of the medium before the tubercle bacillus has had time to form visible colonies, it is usually necessary first to inoculate a guinea-pig, both subcutaneously and intraperitoneally, and then obtain cultures from the animal as soon as the tubercle infection has fully developed. From acute tuberculosis in man in other regions than the lungs direct cultures on blood serum or egg may be made with some hope of success. Under the best conditions great care and patience are necessary if successful results are to be obtained.

Animals inoculated usually die at the end of three weeks to four months. It is better, however, not to wait until death of the animals, but at the end of three weeks to kill a guinea-pig, which by its enlarged glands shows evidence of tuberculosis, and to remove, with the greatest care as to cleanliness, one or more nodules from the lungs, spleen, or lymphatic glands. Animals which develop tuberculosis acutely are apt to have abundant tubercle bacilli and give successful cultures, while the chronic cases usually have few bacilli and frequently give unsuccessful cultures. The animals after being killed are placed in trays, and after washing with a 5 per cent. solution of carbolic acid, immediately autopsied. The skin over the anterior portion of the body having been carefully turned back, an opening is cut with a fresh set of sterile instruments into the thoracic or abdominal cavity; then with a sterile forceps the lymph-gland, portion of spleen or other part which it is desired to examine is removed to a sterile covered beaker. This tissue, if suitable, is sliced in thin sections and conveyed directly to the surface of the solid culture medium. It is allowed to remain in contact with the moist serum or egg at 37° C. for three to ten days, and then by means of a platinum wire gently rubbed and drawn over the surface of the media. The tubes are then replaced in the incubator for ten days to three weeks, when a visible culture should be obtained. Owing to the liability of the blood serum to become too dry for the development of

the bacillus, it is necessary to keep the culture moist by sealing the end in some way. Theobald Smith, who has had a very large experience in growing the tubercle bacillus, gives the following details as to his method:

"Throughout the work solidified dog's serum was used as being the best medium. The dog was bled under chloroform and the blood drawn from a femoral artery, under aseptic conditions, through sterile tubes directly into sterile flasks. The serum was drawn from the clot with sterile pipettes, and either distributed at once into tubes or else stored with 0.25 to 0.3 per cent. chloroform added. The temperature required to produce a sufficiently firm and yet not too hard and dry serum is, for the dog, 75° to 76° C. The tubes containing the serum were set in a thermostat, into which a dish of water was placed, to forestall any abstraction of moisture from the serum. About three hours suffice for the coagulation. This procedure dispenses with all sterilization, excepting that going on during the coagulation of the serum. It prevents the gradual formation of membranes of salts, which, remaining on the surface during coagulation, form a film unsuited for bacteria. Tubes of coagulated serum should be kept in a cold, closed space, where the opportunities for evaporation are slight. They should always be kept inclined.

"The ordinary cotton-plugged test-tubes I do not use, because of the rapid drying out permitted by them as well as the opportunities for infection with fungi. Instead, a tube is used which has a ground-glass cap fitted over it. This cap contracts into a narrow tube plugged with glass-wool; this plug is not disturbed. The tube is cleaned, filled, and inoculated by removing the cap. With sufficient opportunity for the interchange of air very little evaporation takes place, and contamination of the culture is a very rare occurrence. In inoculating these tubes bits of tissue which include tuberculous foci, especially the most recent, are torn from the organs and transferred to the serum. Very little crushing, if any, is desirable or necessary. I think many failures are due to the often futile attempts to break up firm tubercles. Nor should the bits of tissue be rubbed into the surface, as is sometimes recommended. After a stay of several weeks in the thermostat I usually remove the tubes and stir about the bits of tissue. This frequently is the occasion for a prompt appearance of growth of tiny, dull-grayish colonies within a week, as it seems to put certain still microscopic colonies in or around the tissues into better condition for further development. From this first growth of tubercle bacilli fresh serum tubes are inoculated. From these either serum or glycerin-agar tubes are inoculated. The thermostat should be fairly constant, as urged by Koch in his classic monograph; but I look upon moisture as of more importance. If possible a thermostat should be used which is opened only occasionally. Into this a large dish of water is placed, which keeps the space saturated. Ventilation should be restricted to a minimum. As a consequence, moulds grow luxuriantly, and even the gummed labels must be replaced by pieces of stiff manila paper fastened to the tube with a rubber band.

By keeping the tubes inclined no undue amount of condensation of water can collect in the bottom, and the upper portion of the serum remains moist. The only precaution to be applied to prevent infection with moulds is to thoroughly flame the joint between the tube and cap, as well as the plugged end, before opening the tube."

In our experience, when cultures are made exactly according to the above directions, a growth is usually obtained, but Dorset advises the use of an egg medium. Many, including ourselves, have had good results with it. It is more difficult to get a growth of bovine than of the human type of bacilli. All methods frequently fail when the tuberculous tissue used contains very few bacilli.

Pathogenesis.—The tubercle bacillus is pathogenic not only to man, but to a large number of animals, such as the monkey, pig, cow, cat, etc. Guinea-pigs are extremely susceptible, and are much used for the detection of tubercle bacilli in suspected material. When inoculated with the minutest doses of the living bacilli they usually succumb to the disease. Infection is most rapidly produced by intraperitoneal injection. If a large dose is given death follows in from ten to twenty days. The omentum is found to be clumped together in sausage-like masses and converted into hard knots, which contain many bacilli. There is no serous fluid in the peritoneal cavity, but generally in both pleural sacs. The spleen is enlarged, and it, as well as the liver and peritoneum, contains large numbers of tubercle bacilli. If smaller doses are given the disease is prolonged. The peritoneum and interior organs—spleen, liver, etc.—are then filled with tubercles. On subcutaneous injection, for instance, into the abdominal wall, there is a thickening of the tissues about the point of inoculation, which breaks down in one to three weeks and leaves a sluggish ulcer covered with cheesy material. The neighboring lymph glands are swollen, and at the end of two or three weeks may attain the size of hazel-nuts. Soon an irregular fever is set up, and the animal becomes emaciated, usually dying within four to eight weeks. If the injected material contained only a small number of bacilli the wound at the point of inoculation may heal up and death be postponed for a long time. On autopsy the lymphatic glands are found to have undergone cheesy degeneration; the spleen is very much enlarged, and throughout its substance, which is colored dark red, are distributed masses of nodules. The liver is also enormously increased in size, streaked brown and yellow, and the lungs are filled with grayish-white tubercles; but, as a rule, the kidneys contain no nodules. Tubercle bacilli are always found in the affected tissues, but the more chronic the process the fewer the bacilli that are apt to be present.

Rabbits are also quite susceptible to tuberculosis, but considerably less so than guinea-pigs. In rabbits death almost invariably follows inoculations of tuberculous material into the anterior chamber of the eye. The local effects are iris tuberculosis and cheesy degeneration of the pupil. The bacilli then penetrate to the neighboring lymph glands, producing softening of these, then pulmonary tuberculosis, general

tuberculosis, and finally death at the end of several weeks or months. Subcutaneous inoculations are less effective, and in small doses frequently do not kill. Intravenous and intraperitoneal injections usually produce general tuberculosis and death at the end of a few weeks.

Of other susceptible animals, field-mice and cats are readily infected by artificial inoculations of tuberculous material; rats, white mice, and dogs only when very large doses are given. All these animals present the anatomical lesions of miliary tuberculosis. Canaries are also susceptible to inoculations of the tubercle bacillus, but not sparrows. Fowls and pigeons are only moderately susceptible to the bacillus derived from man. Among the larger birds, parrots alone would seem to be clearly susceptible.

Animal Infection by Natural Methods.—Besides the artificial modes of infection referred to, tuberculosis may be caused in animals by *feeding* them with tuberculous material. In this case evidence of infection is usually shown in the mesenteric glands, while the intestinal walls are frequently not affected. Bacilli accompanied by fat much more readily pass through the intestinal mucous membrane or that of the tonsils and pharynx. It is certain that tuberculous infection may be caused, under certain conditions, by absorption through serous or mucous membranes without the evidence of any local lesion.

The experimental production of tuberculosis by *inhalation* of bacilli has been demonstrated by Koch in guinea-pigs, rabbits, rats, and mice, and his results have since been confirmed by many others; but in these experiments the bacilli were usually inhaled in the form of a very thin spray in which they were suspended. The experimental inhalation of dry tuberculous dust has less often proved successful.

Various other tuberculous affections which are natural in man have been produced experimentally in animals, as, for instance, tuberculosis of the joints, tuberculous abscess, etc.

Action upon the Tissues of the Poisons Produced by the Tubercle Bacillus.—Soon after the introduction into the tissues of tubercle bacilli, either living or dead, the cells surrounding them begin to show that some irritant is acting upon them. The connective-tissue cells become swollen and undergo mitotic division, the resultant cells being distinguished by their large size and pale nuclei. A small focus of proliferated epithelioid cells is thus formed about the bacilli, and according to the intensity of the inflammation these cells are surrounded by a larger or smaller number of the lymphoid cells. When living bacilli are present and multiplying the lesions progress, the central cells degenerate and die, and a cheesy mass results, which later may lead to the formation of cavities. Dead bacilli, on the other hand, give off sufficient poison to cause the less marked changes only, and never produce cavities (Prudden and Hodenpyl). Of the gross pathological lesions produced in man by the tubercle bacilli the most characteristic are small nodules, called miliary tubercles. When young, and before they have undergone degeneration, these tubercles are gray and trans-

lucent in color, somewhat smaller than a millet seed in size, and hard in consistence.

But miliary tubercles are not the sole tuberculous products. The tubercle bacilli may cause the diffuse growth of a tissue identical in structure with that of miliary tubercles—that is, composed of a basement substance, containing epithelioid, giant, and lymphoid cells. This diffuse tubercle tissue also tends to undergo cheesy degeneration.

Distribution of Tubercle Bacilli in the Tissues.—In acute tuberculosis, especially when caseation is rapidly spreading, the bacilli are usually abundant. They are generally scattered irregularly through the tissues or in small groups. They are occasionally found in the leukocytes and in the giant and epithelioid cells. In subacute and chronic lesions they are usually few in number. Sometimes in old caseous materials numerous stained granular points are seen; these are supposed by some to be a resting stage similar to spores, but not resistant to heat.

Usual Point of Entrance of Infection.—Infection by the tubercle bacillus takes place usually through the respiratory tract or the digestive tract including the pharynx and tonsils, more rarely through wounds of the skin.

Tuberculosis may be considered to be caused chiefly by the direct transmission of tubercle bacilli to the mouth through soiled hands, lips, handkerchiefs, food, etc., or by the inhalation of fine particles of mucus thrown off by coughing or loud speaking, or of tuberculous dust.

Tuberculosis of Skin and Mucous Membranes.—When the skin or mucous membranes are superficially infected through wounds there may develop lupus, ulceration, or a nodular growth. The latter two forms of infection are apt after an interval to cause the involvement of the nearest lymphatic glands, and then finally the deeper structures.

Tuberculosis of Digestive Tract.—Tuberculosis of the gums, cheeks, and tongue are rare, and usually occur through the germs entering lacerations caused by sharp, ragged teeth. The tonsils and pharynx are somewhat more often involved. The stomach protected through its acid gastric juice, and œsophagus by its epithelium, are almost never attacked. The small intestines, rich in lymph glands, are rather frequently the seat of infection from bacilli swallowed with the food. In a striking case four previously healthy children died within a short period of one another. Their nurse was found to have tuberculosis of the antrum of Highmore, with a fistulous opening into the mouth. She had the habit of putting the spoon with which she fed the children into her mouth so as to taste the food before it was given to them. As already noted, the bacilli frequently pass through the mucous membrane to the lymph glands without leaving any lesions.

Intestinal and mesenteric tuberculosis, which is rather common with children, is due not only to swallowing the bacilli received in the above ways, from human sources, but also to the ingestion of tuberculous milk.

Tuberculosis of Respiratory Tract.—The lungs are the most frequent location of tuberculous inflammation, in spite of the fact that

on account of their location they are greatly protected. Most of the bacilli are caught upon the nasal or pharyngeal mucous membranes. Only a small percentage can find their way to the larynx and trachea, and still less to the smaller bronchioles. From the examination of the lungs of miners as well as from experimental tests there is no doubt but that some of the bacilli find their way into the deeper bronchi. The deeper the bacilli penetrate the more unlikely that they can be cast out. The healthy lung tissue usually destroys these bacilli. The nasal cavities are rarely affected with tuberculosis, but more often the retropharyngeal tissue. Tuberculosis of this tissue as well as that of the tonsils is apt to give rise to infection of the lymphatic glands of the neck. It is believed that just as bacilli may pass through the intestinal walls to infect the mesenteric glands so bacilli may, without leaving any trace, pass through the tonsils to the glands of the neck. It is now well established that infection taking place through various channels may find its way to the lungs and excite there the most extensive lesions.

TUBERCULOSIS OF LARYNX.—Primary infection of the larynx is rare. Secondary infection is fairly common. The region of the vocal cords and the interarytenoid space are the special sites attacked.

INFECTION BY INHALATION OF DRIED AND MOIST BACILLI.—The most common mode of infection is by means of tuberculous sputum, which, being coughed up by consumptives, is either disseminated as a fine spray and so inhaled, or, carelessly expectorated, dries and distributes numerous virulent bacilli in the dust. As long as the sputum remains moist there is no danger of dust infection, but only of direct contact; it is only when it becomes dry, as on handkerchiefs, bedclothes, and the floor, etc., that the dust is a source of danger.

A great number of the expectorated and dried tubercle bacilli undoubtedly die, especially when exposed to the action of direct sunlight; but when it is considered that as many as five billion virulent tubercle bacilli may be expectorated by a single tuberculous individual in twenty-four hours, it is evident that even a much smaller proportion than are known to stay alive will suffice in the immediate vicinity of consumptives to produce infection unless precautions are taken to prevent it. The danger of infection is greatest, of course, in the close neighborhood of tuberculous patients who expectorate profusely and indiscriminately—that is, without taking the necessary means for preventing infection. There is much less danger of infection at a distance, as in the streets, for instance, where the tubercle bacilli have become so diluted that they are less to be feared. In rooms the sputum is not only protected from the direct sunlight, but it is constantly broken up and blown about by the walking, closing of doors, etc. In crowded streets on windy days infected dust must sometimes be in the air unless the expectoration of consumptives is controlled.

Exhaustive experiments made by many observers have shown that particles of dust collected from the immediate neighborhood of consumptives, when inoculated into guinea-pigs, produce tuberculosis in a considerable percentage of them; whereas, the dust from rooms

inhabited by healthy persons or the dusts of the streets does so only in an extremely small percentage. Flügge is probably right in thinking that the dust which is fine enough to remain for a long time in suspension in the air is usually free from living bacilli. It is in the coarser though still minute particles, those in which the bacilli are protected by an envelope of mucus, that the germs resist drying for considerable periods. These are carried only short distances by air currents. Such results as those obtained by Straus, who on examining the nasal secretions of twenty-nine healthy persons living in a hospital with consumptive patients, found tubercle bacilli in nine of them, must be accepted with some reserve, since we know that in the air there are bacilli which look and stain like tubercle bacilli and yet are totally different. It may be said that the danger of infection from slight contact with tuberculosis is not so great as it is considered by many, but that on this account it is all the more to be feared and guarded against in the immediate neighborhood of consumptives. Those who are most liable to infection from this source are the families, the nurses, the fellow-workmen, and fellow-prisoners of persons suffering from the disease. In this connection, also, attention may be drawn to the fact that rooms which have been recently occupied by consumptives are not infrequently the means of producing infection (as has been clinically and experimentally demonstrated) from the deposition of tuberculous dust on furniture, walls, floors, etc. Flügge has recently drawn attention to the fact that in coughing, sneezing, etc., very fine particles of throat secretion are thrown out and carried by air currents many feet from the patient and remain suspended in the air for a considerable time. This is another means of infection, but probably a less frequent one. To encourage us we now have a mass of facts which go to show that when the sputum is carefully looked after there is very little danger of infecting others except by close personal contact.

Individual Susceptibility.—It is believed by many that in demonstrating the possibility of infection in pulmonary tuberculosis its occurrence is sufficiently explained; but they leave out another and most important factor in the production of an infectious disease—individual susceptibility. That this susceptibility, or "predisposition," as it is improperly called, may be either inherited or acquired is now an accepted fact in medicine. It is even thought that the physical signs and characters—the *phthisical habit*—which indicate this susceptibility can be externally recognized. At first the inherited susceptibility was considered more important than the acquired, but now much that was attributed to the former is known to be explained by the fact of living in an infected area. The acquired susceptibility may arise from faulty physical development or from depression, sickness, overwork, excessive use of alcohol, etc. Unquestionably, vast differences exist in different individuals in the intensity of the tuberculous process in the lung. That this does not depend chiefly upon a difference in virulence of the infection is evident, from the fact that individuals contracting tuberculosis from the same source are attacked with different

severity, and that there is, as a rule, no great difference in degrees of virulence for animals in the tubercle bacilli obtained from different sources. As is seen from the results of post-mortem examinations in which, according to the completeness of the examinations, the remains of old tuberculous processes have been found in the lungs of about one-third to one-half of all the bodies examined, many cases of pulmonary infection must occur without showing any visible evidence of disease, and heal of their own accord. The possibility of favorably influencing in many an existing tuberculosis by treatment also proves that, under natural conditions, there is a varying susceptibility to the disease. Clinical experience teaches, likewise, that good hygienic conditions, pure air, good food, freedom from care, etc., increase immunity to phthisis. Animal experiments have shown that not only are there differences of susceptibility in various animal species, but also an individual susceptibility in the same species. The doctrine of individual susceptibility, therefore, is seen to be founded on fact, although the reasons for it are only partially understood.

Infection by Ingestion of Milk and Meat.—Phthisical sputum, however, is not held responsible for the occurrence of all human tuberculosis. Milk also serves as a conveyer of infection, whether it be the milk of nursing mothers suffering from consumption or the milk of tuberculous cows. The transmission of tubercle bacilli in the milk of tuberculous cows has been abundantly proved. Formerly it was thought that in order to produce infection by milk there must be a local tuberculous affection of the udder; but it is now known that tubercle bacilli may be found in the milk when an internal organ is infected, and when careful search fails to detect any udder disease. The milk of every cow which has any well-developed, internal, tuberculous infection must therefore be considered as possibly containing tubercle bacilli. Rabinowitsch and Kempner proved beyond all question that not only the milk of tuberculous cattle, which showed no appreciable udder disease, but also those in which tuberculosis was only detected through tuberculin, frequently contained tubercle bacilli. Different observers have found tubercle bacilli in the milk of from 20 to 60 per cent. of tuberculous cows. When we consider the prevalence of tuberculosis among cattle we can readily realize, even if the bovine bacillus with difficulty infects human beings, the danger to which children are exposed from this source of infection. Thus, taking the abattoir statistics of various countries; we find that about 10 per cent. of the cattle slaughtered were tuberculous. A less probable source of infection by way of the intestines is the flesh of tuberculous cattle. Here the danger is considerably less, from the fact that meat is usually cooked, and also because the muscular tissues are seldom attacked. In view of the finding of the bovine type of bacilli in a considerable percentage of the few cases tested of tuberculous children, the legislative control and inspection of cattle and milk would seem to be an absolute necessity. As a practical and simple method of preventing infection from suspected milk the sterilization (by heat) of the milk used as food must

commend itself to all. It is only right to state, however, that up to the present time the actual proof that human tuberculosis has frequently come from milk or food infected with bovine tuberculosis is very small, and that it is probable that the bovine bacilli are not as virulent for man as for animals. The relation of bovine to human tubercle bacilli will be discussed more fully later.

AUTOINFECTION BY SWALLOWING SPUTUM.—The *secondary* forms of tuberculosis which often succeed a primary infection of the lungs may be explained as autoinfections, from the coughing up and swallowing of sputum containing bacilli. It is a wonder, indeed, that intestinal tuberculosis is not more common than it is in consumption; but this is probably due to the action of the gastric juice and to the fact that in adults the intestines are comparatively insusceptible.

Hypothesis of Transmissibility of Tubercle Bacilli to the Fœtus.—There is some evidence of the transmission of tubercle bacilli from the mother to the fœtus in animals. The first authentic case recorded is that reported by Johne of an eight-months-old calf fœtus; other cases have since been reported. With regard to tuberculosis in the human fœtus the evidence is not so clear, though some twenty cases have been reported of tuberculosis in newly born infants, and about a dozen cases are recorded of placental tuberculosis. The fact that statistics show a greater frequency of tuberculous diseases in children during the first than in the following years of life, does not strengthen the hypothesis of frequent infection *in utero;* for nursing infants would naturally be more exposed to infection through the mother's milk and through personal contact than others. According to experiments upon laboratory animals one would expect to find in man fetal or placental tuberculous infection more common than it is, whereas it is extremely rare, even if the few cases reported be accepted as proven. Possibly the few bacilli which may be transmitted to the fœtus do not find conditions favorable for their development, and, being so few in number, die; or they may remain latent, as has been suggested by Behring and others, for certain lengths of time without producing visible effects, and only show symptoms of infection later; but we have no experimental confirmation of any such latency existing with regard to the tubercle bacillus, and it is not to be assumed that it does exist. As to the infection of the fœtus from the paternal side, where the father has tuberculosis of the scrotum or seminal vessels (which have been found to be tuberculous in exceptional cases), we have no reason to suppose that such ean occur. There are, however, grounds for belief that infection in this way may take place from husband to wife. Thus, Gärtner found, as a result of his experiments in animals, that a large majority of the guinea-pigs and rabbits which were brought together with males whose semen contained tubercle bacilli died of primary genital tuberculosis; but from the rarity of this affection in women and cows it may be assumed that tubercle bacilli occur very much less frequently in semen of men and cattle than in that of the smaller animals. It is believed that the semen of those suffering from advanced or local genital tuberculosis

contain tuberculous toxins which cripple the activity of the spermatozoa.

Length of Time Tubercle Bacilli Remain Virulent in Sputum.—According to experimental investigations, the virulence of dried tuberculous sputum is not suddenly but gradually lost, a certain proportion of it retaining its specific infective power under ordinary conditions, as in a dwelling-room, for at least two or three months, and occasionally for a year or more.

Attenuation.—Tubercle bacilli when subjected to deleterious influences or to growth on culture media gradually lose their virulence. A culture which Trudeau has grown on suitable media for ten years is no longer capable of causing tuberculosis in healthy guinea-pigs, although originally virulent. Cultures grown at temperatures of 42° C. become attenuated more quickly.

Mixed Infection.—In regions where tuberculous processes are on the surface, such as lung and skin infections, and also when the infection itself is multiple, as in disease of the glands of the neck from tonsillar absorption, the tubercle bacilli are usually associated with one or more other varieties of organisms. Those of most importance are the streptococcus, pneumococcus, and influenza bacillus. Besides these many other varieties are met with occasionally in individual cases. What the influence of this secondary or mixed infection is, under all circumstances, is not exactly known; but generally the effect is an unfavorable one, and not infrequently after a time the disease takes on a septicæmic character. For the technique employed in examining sputa for mixed infection see page 312.

Immunization.—As in other infectious diseases, many attempts have been made to produce an artificial immunity against tuberculosis, but so far the results have been only fairly satisfactory. The great majority of mankind has in a varying degree a natural immunity against tuberculosis. In many individuals this immunity is only relative, and is maintained only so long as the health is kept at a high standard or the exposure to infection not too intense or prolonged. An unfavorable environment, the occurrence of some other infectious disease, overwork, dissipation, or, in fact, anything which tends to depreciate the nutrition of the body, is apt to render the individual previously immune susceptible to the tubercle bacillus.

Acquired immunity against many bacterial diseases is acquired within a few days or weeks after the development of infection. This immunity may be complete or slight and vary greatly in its duration. There is little in the clinical history of tuberculosis which shows that acquired immunity occurs in this disease, for relapse is the rule, and one attack does not seem to afford any protection against a later one. For this reason the production of an artificial immunity against tuberculosis has always been looked upon as a result possibly never to be achieved. The careful study of tuberculosis seems, however, to indicate an attempt on the part of nature at the production of acquired immunity in this disease. It is thought that from 30 to 60

per cent. of cadavers show the healed lesions of tuberculosis. The small proportion of these which progressed to serious lesions or became reinfected indicate a degree of acquired immunity. Artificial immunity is an attempt to imitate nature's methods, and is obtained by the inoculation of a modified living culture or of toxins and dead bacteria. The injection of toxins, as in Koch's tuberculin treatment, produces in animals a certain degree of acquired resistance to larger doses of toxins, but does not protect to any appreciable degree from subsequent living tubercle bacilli, or produce in animals an antitoxic serum. In 1892 Trudeau succeeded in producing in rabbits an appreciable immunity by inoculations of living avian cultures. The rabbits so treated supported, as a rule, inoculation of virulent tubercle bacilli in the anterior chamber of the eye, while in controls the eyes were invariably lost. Later, attenuated human cultures were used with the same results. De Schweinitz, McFadyan, Behring, and Pearson Gilliland have since reported successful results. The latter two treated a number of cows by giving each of them seven intravenous injections of 1 to 6 c.c. of an emulsion of tubercle bacilli. This was of an opacity equal to a twenty-four-hour broth culture of typhoid bacilli. They report from their investigations[1] that the treatment had the effect not only in keeping in check the progress of the tuberculous process, but of causing in some cases a distinct retrogression. The bacilli remained alive in the encapsulated lesions.

The work already done is believed by Trudeau to establish the principle that in order to be successful the protective inoculation must be made with living germs of such diminished virulence for the animal experimented upon as to produce a reaction ending in healing of the process at first set up by them. This is termed by Behring isopathic immunity.

The avian and bovine bacilli immunize against infection from human bacilli equally as well as the attenuated human variety. This is strong evidence in favor of the genetic unity of all tubercle bacilli. Up to the present time the results in animals hardly permit the inoculation of man with living bacilli for purposes of producing immunity. The practical difficulties which confront one make it at present probably unadvisable to use such methods in cows except in an experimental way.

The serum of animals treated with bacilli and their products has not given curative results.

Among the numerous medicinal agents that have been tried without avail to protect animals against the action of the tubercle bacillus may be mentioned tannin, menthol, sulphuretted hydrogen, mercuric chloride, creosote, creolin, phenol, arsenic, eucalyptol, etc.

Agglutination.—The results obtained by various observers has been very conflicting. Two methods are employed in making the test. In one a vigorous growth of bacilli is dried, ground up and an emulsion

[1] University of Pennsylvania Medical Bulletin, April, 1905.

made. In the other Arloing and Courmont grow the culture for a time on potato and then in bouillon. In this way a homogeneous culture of separate bacilli is obtained which can be used for agglutination. The examination is usually made macroscopically, and requires twelve to twenty-four hours. At present the test cannot be advised as useful in diagnosis as the sera of cases suffering from tuberculosis frequently fail to give a reaction, while the sera from those having no detectable tuberculosis frequently cause a good reaction. A positive agglutination test in tuberculosis seems to be a favorable sign as indicating resistance to infection by the body.

Chemical Constituents of Tubercle Bacilli.—The bacilli contain on an average 86 per cent. water. The dry substance consists of material soluble in alcohol and ether, of proteid substance extracted by warm alkaline solutions, and of carbohydrates and ash. The alcohol-ether extract equals about one-quarter of the dry substance and consists of 15 per cent. of a fatty acid, which is mostly combined with an alcohol to make a wax. No glycerin is present and, therefore, no true fat. It is on the presence of this wax that the staining characteristics depend. Other substances produce abscess, necrosis, and cheesy degeneration. Lecithin and a convulsive poison are also present in the extract.

The substances left after the ether-alcohol extraction are mostly proteid substances. A nucleic acid which contains phosphorus is present which, according to Behring, is the specific principle of tuberculin.

Tuberculin (Koch's).—Tuberculin contains not only the products of the growth of the tubercle bacilli in the nutrient bouillon which withstand heat as well as substances extracted from the bodies of the bacilli themselves, but also the materials originally contained in the bouillon, which have remained unaffected by the activities of the bacilli. There are two preparations known respectively as the old and the new tuberculin.

Old tuberculin is prepared as follows: The tubercle bacillus is cultivated in an infusion of calf's flesh or of beef flesh, or extract to which 1 per cent. of peptone and 4 to 5 per cent. of glycerin have been added, the culture liquid being slightly alkaline. The inoculation is made upon the surface from a piece of very thin pellicle from a young bouillon culture, or, if the bouillon culture is unobtainable, with small masses from a culture on glycerin agar. These masses, floating on the surface, give rise in from three to six weeks, according to the rapidity with which the culture grows, to an abundant development and to the formation of a tolerably thick and dry, white crumpled layer, which finally covers the entire surface. At the end of four to eight weeks development ceases, and the layer after a time sinks to the bottom. Fully developed cultures, after having been tested for purity by a microscopic examination, are passed into a suitable vessel and evaporated to one-tenth of their original bulk over a water-bath at a temperature of 70° to 80° C. The liquid is then filtered through chemically pure sterilized filter paper. The crude tuberculin thus obtained contains 40 to 50 per cent. of glycerin, 10 per cent. of albumoses, traces of peptone, extractives,

and inorganic salts. The true nature of the toxic substances is not known. It keeps well, retaining its activity indefinitely.

The method of treatment and the results obtained from the old tuberculin have been described by Koch briefly as follows: After each injection, which should be large enough to cause a slight but not a great rise of temperature, a noticeable improvement in the tuberculous process results. The amount of tuberculin injected is continually increased, so as to continue the moderate reactions. After several months all reactions cease, the patients having become temporarily immune to the toxin, but not to the growth of the bacillus. Further injections are now useless until this immunity has passed. During the treatment the bacilli themselves have not been directly affected, and when the treatment is interrupted the tuberculous process is apt to progress. Some cases, however, of pure tuberculosis of moderate extent become cured or greatly benefited by several periods of treatment. When the seat of tuberculous lesions is visible, as in lupus, a moderate dose of tuberculin causes a visible inflammatory reaction, which may result in necrosis and a casting off of the infected tissue. The bacilli themselves are not killed.

According to Koch, the substances produced in the body by the old tuberculin neutralized the tuberculous toxins, but were not bactericidal. After a series of experiments he considered the difficulty to be due to the nature of the envelope of the tubercle bacillus, which made it difficult to obtain the substance of the bacilli in soluble form without so altering it by heat or chemicals that it was useless to produce immunizing substances. He conceived that immunity was not produced in man for somewhat similar reasons—possibly the bacilli never giving out sufficient toxin to cause curative substances to be produced. He therefore decided to grind up the dried bacilli and soak them in water, and thus obtain, if possible, without the addition of heat, a soluble extract of the body substance of the bacilli, which he hoped would be immunizing. He also tried to eliminate as much as possible of the toxic products which produce fever. Buchner by a different method, through crushing under a great pressure tubercle bacilli mixed with sand, and thus squeezing out their protoplasm, obtained a very similar substance.

The *new tuberculin* formed by either of these methods is a watery extract of the soluble portions of the unaltered tubercle bacilli. As can be readily seen, in a preparation thus made, contamination is difficult to avoid, freedom from intact bacilli is uncertain, and the strength of the solution prepared at different times is variable. Twenty per cent. of glycerin is added to preserve the tuberculin from contamination. After three years of trial the results obtained with the new tuberculin preparations cannot be considered to have exerted either very different or very superior effects to the older product.

As to the results obtained in general the reports are as yet conflicting. Lupus seems to be decidedly benefited for a time both by the old and the new tuberculin. Relapses are, however, common. On advanced

phthisis, laryngeal tuberculosis, and other tuberculous processes no effects have been noted, and nearly everyone disapproves of their use in these cases as well as in those where mixed infection is suspected; even in cases of beginning infection, opinions, as a whole, are not very enthusiastic. The new tuberculin, unless prepared with great care or from tubercle bacilli, which are non-virulent for man, is apt to be a dangerous substance. Trudeau, Baldwin, and others found that with the first product sent out guinea-pigs injected with it not only did not become immunized, but actually became infected from the living bacilli in the fluid.

Diagnostic Use of Tuberculin.—The chief use to which tuberculin has been put is as an aid to the diagnosis of tuberculosis in cattle and human beings, and for this purpose it has proved to be of inestimable value. Numerous experiments made by veterinary surgeons show that the injection of tuberculin in tuberculous cows in doses of 25 to 50 centigrams produces in at least 95 per cent. a rise of temperature of from 1° to 3° C. (2° to 5° F.). The febrile reaction occurs in from twelve to fifteen hours after the injection. Its intensity and duration do not entirely depend upon the extent of the tuberculous lesions, being even more marked when these are slight than in advanced cases. In non-tuberculous animals no reaction occurs, or one much less than in tuberculous animals, and the results obtained on autopsy justify the suspicion that tuberculosis exists if an elevation of temperature of a degree or more centigrade occurs and remains for ten hours from the subcutaneous injection of the dose mentioned. It must always be remembered that cattle may have a rise of temperature from other conditions, and it is only when due to tuberculin that infection is proved. When properly carried out an error of more than 5 per cent. is impossible. For these injections the original tuberculin is used, which for the convenience of administration is diluted with water.

United States Government Directions for Inspecting Herds for Tuberculosis.—"Inspections should be carried on while the herd is stabled. If it is necessary to stable animals under unusual conditions or among surroundings that make them uneasy and excited, the tuberculin test should be postponed until the cattle have become accustomed to the conditions they are subjected to, and then begin with a careful physical examination of each animal. This is essential, because in some severe cases of tuberculosis, on account of saturation with toxins, no reaction follows the injection of tuberculin, but experience has shown that these cases can be discovered by physical examination. This should include a careful examination of the udder and of the superficial lymphatic glands, and auscultation of the lungs.

"Each animal should be numbered or described in such a way that it can be recognized without difficulty. It is well to number the stalls with chalk and transfer these numbers to the temperature-sheet, so that the temperature of each animal can be recorded in its appropriate place without danger of confusion. The following procedure has been used extensively and has given excellent results:

"(*a*) Take the temperature of each animal to be tested at least twice, at intervals of three hours, before tuberculin is injected.

"(*b*) Inject the tuberculin in the evening, preferably between the hours of six and nine. The injection should be made with a carefully sterilized hypodermic syringe. The most convenient point for injection is back of the left scapula. Prior to the injection the skin should be washed carefully with a 5 per cent. solution of carbolic acid or other antiseptic.

"(*c*) The temperature should be taken nine hours after the injection, and temperature measurements repeated at regular intervals of two or three hours until the sixteenth hour after the injection.

"(*d*) When there is no elevation of temperature at this time (sixteen hours after the injection) the examination may be discontinued; but if the temperature shows an upward tendency, measurements must be continued until a distinct reaction is recognized or until the temperature begins to fall.

"(*e*) If a reaction is detected prior to the sixteenth hour, the measurements of temperature should be continued until the expiration of this period.

"(*f*) If there is an unusual change of temperature of the stable, or a sudden change in the weather, this fact should be recorded on the report blank.

"(*g*) If a cow is in a febrile condition tuberculin should not be used, because it would be impossible to determine whether, if a rise of temperature occurred, it was due to the tuberculin or to some transitory illness.

"(*h*) Cows should not be tested within a few days before or after calving, for experience has shown that the result at these times may be misleading.

"(*i*) The tuberculin test is not recommended for calves under three months old.

"(*j*) In old, emaciated animals and in re-tests, use twice the usual dose of tuberculin, for these animals are less sensitive.

"(*k*) Condemned cattle must be removed from the herd and kept away from those that are healthy.

"(*l*) In making post-mortems the carcasses should be thoroughly inspected, and all of the organs should be examined."

Tuberculin injections are also made in man to reveal a suspected tuberculosis. At first some believed that the irritation aroused in the tuberculous foci by the tuberculin sometimes caused a dissemination of the bacilli and an increase in the disease. When carefully used, however, in suitable cases there is probably no danger. A drawback to its usefulness is that it does not reveal the extent of the disease, nor whether the tuberculosis is active. It is, however, of great value in selected cases, both surgical and medical, where slight tuberculosis is suspected, and yet no decision can be reached. In the small doses advised an absolutely latent infection would probably give no rise of temperature. I quote here Dr. Trudeau upon the use of the test:

"The range of the patient's temperature is ascertained by taking it at 8 A.M., 3 P.M., and 8 P.M. for three or four days before making the test. The first injection should not exceed 0.5 mg., and if any fever is habitually present should be even less, and is best given early in the morning or late at night, as the typical reaction usually begins, in my experience, within six or twelve hours. Such a small dose, while it will often be sufficient to produce the looked-for rise of temperature, has, under my observation, never produced unpleasant or violent symptoms. An interval of two or three days should be allowed between each of the two or three subsequent injections it may be necessary to give, as reaction in very rare cases may be delayed for twenty-four or even thirty-six hours. On the third day a second dose of 1 mg. is given, and if no effect is produced a third, of 2 mg., three days later. In the great majority of cases of latent tuberculosis an appreciable reaction will be produced by the time a dose of 2 mg. has been reached. If no effect has been caused by the tests applied as above I have usually gone no farther, and concluded that no tuberculous process was present, or at least not to a degree which need be taken into account in advising the patient, or which would warrant insisting on a radical change in his surroundings and mode of life. If some slight symptoms, however, have been produced by a dose of 2 mg., it may be necessary to give a fourth injection of 3 mg. in order to reach a positive conclusion. Nevertheless, it should be borne in mind that in a few cases the exhibition of even larger doses may cause reaction and indicate the existence of some slight latent tuberculous lesion, and the test should not, when applied within the moderate doses described, be considered absolutely infallible.

"No evidence in connection with the tuberculin test as applied to man and animals has been forthcoming thus far from those who have made use of it, which would tend to sustain the general impression that this method is necessarily dangerous and tends invariably to aggravate the disease, and my own experience has developed nothing which would seem to confirm this impression. It is evident that the size of the doses given has much to do with the limitations of this method for usefulness, and the correctness of the conclusions reached by its application. The tuberculin used is also a matter of some importance in determining the dosage, as different samples vary considerably in their efficiency. If the test be pushed to the injection of such large amounts as 10 mg. or more, as advocated by Maragliano, such doses are by no means free from the objection of occasionally causing unpleasant and sometimes dangerous symptoms; and even if the amount given be not carried to the dose of 10 mg., which is known to produce fever in healthy subjects, it is likely that on account of individual susceptibility or the presence of some other morbid process in the body, reaction will be found to occur with the larger doses when no tuberculous process exists. The adoption of an initial dose so small as to guard against the absolute possibility of producing violent reactionary symptoms, and the graded increase of the subsequent doses within

such quantities as are known never to produce reaction in healthy individuals, would seem to afford the best protection against unpleasant results and misleading evidence."

Antituberculous Serum.—Whether serum therapy is destined to solve the problem of the treatment of tuberculosis remains for the future to decide, but up to the present the results obtained with antituberculous serum do not warrant our forming such an opinion. Every conceivable way of obtaining the true products of the tubercle bacilli has been tried, so as to cause the injected animals to produce antibodies both antitoxic and bactericidal. At present Maragliano and Marmorek are presenting claims that their sera are truly curative. Although both these men have had a large experience in this field of investigation, it is probable that the final judgment will be that little good comes from the injection of their serum. Very few observers have succeeded in obtaining appreciable results with the serums prepared by other experimenters. In spite of much conflicting testimony, it is probably safe to assert that no sera now obtainable have any great value. Nor as we look at the progressive nature of tuberculosis can we see much ground to hope for the abundant development of curative substances in the blood of animals. This view, however, in no way lessens the necessity of continued endeavor until every method conceivable has been tried.

Prophylaxis.—Meanwhile all energies should be directed to the prevention of tuberculosis, not only by the enforcement of proper sanitary regulations as regards the care of sputum, milk, meat, disinfection, etc., but also by continued experimental work and by the establishment of free consumptive hospitals, and by efforts to improve the character of the food, dwellings, and condition of the people in general, we should endeavor to build up the individual resistance to the disease. It may be years yet before the public are sufficiently educated to co-operate with the sanitary authorities in adopting the necessary hygienic measures to stamp out tuberculosis entirely; but, judging from the results which have already been obtained in reducing the mortality from this dread disease, we have reason to believe that in time it can be completely controlled.

The Tubercle Bacillus of Cattle and its Relation to Human Tuberculosis.—Among the domestic animals tuberculosis is most common in cattle. On account of the milk which they provide for our use, and which is liable to contain bacilli, the relation of these to human tuberculosis is a matter of extreme importance.

The chief seat of the lesions is apt to be the lungs, and with them the pleura; less often the abdominal organs and the udder are affected. In pigs and horses the abdominal organs are most often involved, then the lungs and lymphatic glands. In sheep and goats tuberculosis is rare. The bovine bacillus, as the most important of the group, will be alone considered here.

The bacilli derived from cattle are on the average a little shorter and straighter than the average human bacillus. In guinea-pigs, and espe-

cially in rabbits, the bovine bacilli are more virulent than the majority of those from human sources. Animals infected with the bacilli from cattle, as well as those from the other domestic animals, react to the tuberculin test. All these bacilli are, therefore, undoubtedly from the same original stock, and at first glance we might consider it unnecessary to prove that those derived from cattle were capable of causing human tuberculosis. There are facts, however, which have made this investigation of great importance. As we investigate we find that all facts tend to show that the great majority of cases of tuberculosis in human adults come from human infection. The cases where fairly strong proof of bovine infection has been obtained are certainly rare.

Further, we have the undoubted fact that the long sojourn of bacteria in one species of animal tends to increase the virulence of the germ for that animal and to lessen it for others.

Theobald Smith has made the interesting discovery that there is a wide difference between the culture growth of the average bovine bacillus and the average one from human sources; the bovine bacilli when grown in glycerin broth causes the broth to become less and less acid and finally feebly alkaline to phenolphthalein, while the human types cause it to become only a little less acid, but never alkaline. In ordinary peptone bouillon the reaction cure is the same for both. The broth becomes alkaline. Tuberculin made from bovine cultures is alkaline while that made from human cultures is markedly acid. Both cultures act upon the glycerin, but in different ways. He had previously noted that the bovine bacilli in cultures were shorter and straighter, and grew less luxuriantly than those from man, and, further, that the bovine bacilli were much more virulent for rabbits. He has found these differences persist for long periods, and believes that the simple passage through a single person in a case of human tuberculosis would not be sufficient to change these characteristics. He has had a chance to examine the bacilli of two cases in young children which were of the bovine type, but in adults not one of some half a dozen cultures showed the bovine characteristics. The proof that Theobald Smith gathered, which proved the difference between the bovine and human bacilli, has enabled him and others to conclusively prove that a considerable proportion of children suffering from intestinal or mesenteric tuberculosis are really infected with bovine bacilli. These bacilli are capable of infecting cattle.

At present then we must assume that bovine bacilli are capable of infecting those who are very susceptible, such as young children. Whether adults are also infected has not yet been decided. Such views as Behring's, that much adult tuberculosis is due to infection in childhood with bovine bacilli which have remained latent, are probably very extreme. This question is in great need of further study. At present no cattle which are tuberculous should be allowed to furnish milk, or at least such milk should not be used for drinking purposes without being sterilized. The flesh is less harmful, as muscular tissue is seldom infected.

Bird (Avian) Tuberculosis.—Tuberculosis is very common and infectious among fowls. The bacilli themselves grow more readily on artificial culture media and produce a more even and moist growth. They are able to develop at a temperature of 43.5° C., which is above that at which the human and bovine types can usually grow. The bacilli are more apt to show branching forms than the human. In rabbits they produce very similar lesions, but guinea-pigs are much less susceptible. Birds are much less susceptible to bacilli from human or bovine sources than to those from birds. Nocard states that bacilli from human sources placed in collodion sacs and inserted into the peritoneal cavity of birds gradually acquire avarian characteristics, so that after eighteen months they can readily infect fowls and approach the avian cultural type. This suggests that bovine bacilli remaining in man for years might acquire human-type characteristics. They are undoubtedly from the same stock as the mammalian varieties, but have become modified; it is not believed that they are any factor in the production of human tuberculosis.

Tuberculosis in Fish.—In certain species of fish a tuberculous disease has been noted. The bacilli have the staining characteristics of the warm-blooded types, but do not grow at body temperature and do not affect mammals.

Methods of Examination for Tubercle Bacilli and Other Associated Bacteria.

One of the most important results of the discovery of the tubercle bacillus relates to the practical diagnosis of tuberculosis. The staining peculiarities of this bacillus renders it possible by the bacteriological examination of microscopic preparations to make an almost absolutely positive diagnosis in the majority of cases. A still more certain test in doubtful cases is the subcutaneous or intraperitoneal injection of guinea-pigs, which permits of the determination of the presence of numbers of bacilli, so small as to escape detection by microscopic examination. For the animal test, however, time is required—at least three weeks, and, when the result is negative, at least six weeks—before any positive conclusion can be reached, for when only a few bacilli are present tuberculosis develops slowly in animals.

Microscopic Examination of Sputum for the Presence of Tubercle Bacilli.
1. Collection of Material.—The sputum should be collected in a clean bottle (two ounce) with a wide mouth and a water-tight stopper, and the bottle labelled with the name of the patient or with some other distinguishing mark. The expectoration discharged in the morning is to be preferred, especially in recent cases, and the material should be coughed up from the lungs. Care should be taken that the contents of the stomach, nasopharyngeal mucus, etc., are not discharged during the act of expectoration and collected instead of pulmonary sputum. If the expectoration be scanty the entire amount discharged in twenty-four hours should be collected. In pulmonary tuberculosis the puru-

lent, cheesy, and mucopurulent sputum usually contains bacilli; while pure mucus, blood, and saliva, as a rule, do not. When hemorrhage has occurred, if possible, some purulent, cheesy, or mucopurulent sputum should be collected for examination. The sputum should not be kept any longer than necessary before examination, for, though a slight delay or even till putrefaction begins, does not vitiate the result so far as the examination for tubercle bacilli is concerned, it almost destroys any proper investigation of the mixed infection present; it is best, therefore, to examine it in as fresh a condition as possible, and it should be kept on ice until examined if cultures are to be made.

2. METHODS OF EXAMINATION. *Examination for Tubercle Bacilli.*—Pour the specimen into a clean, shallow vessel, having a blackened bottom—a Petri dish placed upon a sheet of dull black paper answers the purpose—and select from the sputum some of the true expectoration, containing, if possible, one of the small white or yellowish-white cheesy masses or "balls" which it contains. From this make rather thick cover-glass or slide "smears" in the usual way. In doubtful cases a number of these coarse or fine particles should be placed on the slide. The material being thick, should be evenly spread and very thoroughly dried in the air before heating. Immerse this in a *solution of Ehrlich's aniline-water* fuchsin, contained in a thin watch-glass or porcelain dish, and steam over a small flame for two minutes. Then remove the glass from this and wash with water. Now decolorize by immersing the stained preparation in a 3 per cent. hydrochloric acid solution in alcohol for from one-half up to one minute, removing at the time when all color is just about gone from the cover-glass smear. Wash thoroughly with water, and make a contrast stain by applying a cold solution of Loeffler's alkaline methylene blue—

Concentrated alcoholic solution of methylene blue . .	30 c.c.
Caustic potash (1:10,000 solution)	100 "

for from fifteen to thirty seconds. Wash with water; press between folds of filter paper; dry in the air; mount and examine.

The tubercle bacilli are distinguished by the fact that they retain the red color imparted to them in the fuchsin solution, while the other bacteria present, having been decolorized in the acid solution, take the contrast stain and appear blue. (See Plate I., Figs. 1 and 2.)

Various methods have been suggested for the staining of tubercle bacilli, but the original method as employed by Koch, or some slight modification of it, is so satisfactory in its results that it seems unnecessary to substitute others for it. The above is a slight modification of the Koch-Ehrlich method, differing from it chiefly in the use of a weak for a strong acid decolorizer. It has been found that the strong acid solution originally employed (5 per cent. sulphuric acid solution in alcohol) often decolorizes some of the bacilli entirely by its too energetic action, and that a weaker decolorizer, such as the above, gives more uniform results.

Instead of the Koch-Ehrlich aniline-water solution, *Ziehl's carbol-fuchsin solution* may be used, and is by many preferred. Instead of floating the cover-glass smears on the staining fluid they can be held in the Cornet forceps, covered and kept covered completely with fluid while steamed for two minutes over the flame.

The Koch-Ehrlich aniline-water solution decomposes after having been made for a time, so that it must be freshly prepared as needed. Solutions older than fourteen days should not be used. The advantages in using Ziehl's carbol-fuchsin solution are that it keeps well and is more convenient for use in small quantities.

Another method, which is often of value on account of its simplicity and rapidity of performance, is that of Fränkel as modified by Gabbett. This consists in staining the cover-glass "smear" with steaming Ziehl's carbol-fuchsin solution for from one to two minutes, and then, after washing in water, placing it from one-half to one minute directly in a second solution which contains both the acid for decolorizing and the contrast stain. This second solution consists of—

Sulphuric acid	25 c.c.
Methylene blue in substance	2 grm.
Water	75 c.c.

It is then washed with water and is ready for examination. The tubercle bacilli will remain red as stained by the fuchsin, while all the other bacteria will be tinted blue.

When the number of tubercle bacilli in sputum is very small they may easily escape detection. Methods have, therefore, been suggested for finding them under these circumstances. Ribbert proposed the addition to the sputum of an equal amount or more of a 2 per cent. solution of caustic potash, and boiling the mixture. The mucus is stirred slightly until it is dissolved. To this an equal amount of water is stirred in and the whole is placed in a conical glass vessel; and any bacilli present are deposited at the bottom and may be found in the sediment after removing the supernatant fluid. The sedimentation may be obtained more quickly by the centrifugal machine.

Detection of Tubercle Bacilli in Urine and Feces.—The catheterized urine is centrifuged. If little sediment appears, the upper portion of the fluid is removed and the remainder again centrifuged. If the urine is rich in salts of uric acid, the same may be diminished by carefully warming the urine before treating it. If too alkaline add a little acetic acid.

The feces are examined for any purulent or mucous particles. If none are found the larger masses of feces are removed and then the rest diluted and centrifugalized. The examiner must remember that bacilli swallowed with the sputum may appear in the feces.

Examination for Other Bacteria (Mixed Infection).—With regard to the *bacteriological diagnosis* of pulmonary phthisis, many consider that it is not enough to show only the presence of tubercle bacilli; it is held to be of importance, both for purposes of prognosis and treatment, that the presence of other micro-organisms which may be associated

with the tubercle bacillus should also be determined. It is now usual to distinguish pure tuberculosis of the lungs from a mixed infection. Phthisis due to the tubercle bacillus alone, which constitutes but a small percentage of all cases, may occur almost without febrile reaction; or when fever occurs the prognosis is unfavorable, thus indicating that the disease is already advanced. It is in the uncomplicated forms of phthisis, moreover, where one must expect if anywhere the best results from treatment with tuberculin or antituberculous serum. The majority of cases, however, of pulmonary tuberculosis show a mixed infection, especially with varieties of the streptococcus and pneumococcus. These cases may be active, with fever, or passive, without fever, according, perhaps, as the parenchyma of the lung is invaded by the bacteria; or they are only superficially located in cavities, bronchi, etc. Mixed infection with the staphylococcus, other micrococci, and with the influenza bacilli have also been frequently met with by us. The tetragenus has not been often detected by us in thoroughly washed fresh sputum. At present the facts seem to prove that the tubercle bacilli have in the great majority of cases, at least until shortly before death, a much more important role than the associated bacteria.

Sputum Washing.—Some of the associated bacteria found in the expectoration come from the diseased areas of the lungs, while others were merely added to the sputa as it passed through the mouth, or have developed after gathering. To endeavor to separate the one from the other we wash the sputa. The first essential is that the material be washed within a few minutes, and certainly within an hour, of being expectorated. If a longer time is allowed to intervene, the bacteria from the mouth will penetrate into the interior of the mucus, and thus appear as if they came from the lungs. Sputum treated twenty-four hours after its expectoration is useless for examining for anything except the tubercle bacillus. A rough method is to pour some of the specimen of sputum to be examined into a convenient receptacle containing sterile water, and withdraw, by means of a sterilized platinum wire, one of the cheesy masses or thick "balls" of mucus. Pass this loop five times through sterile water in a dish; repeat the operation in fresh water in a second and third dish. Spread what remains of the mass on cover-glasses and make smear preparations; stain and examine. With another mass inoculate ascitic bouillon in tubes and agar in plates.

When we wish to thoroughly exclude mouth bacteria, a lump of the sputum raised by a natural cough is seized by the forceps and transferred to a bottle of sterile water and thoroughly shaken; it is then removed to a second bottle of bouillon and again thoroughly shaken. From this it is passed in the same way through four other bottles of bouillon. A portion of the mass is now smeared over cover-glasses, and the rest inoculated in suitable media, such as agar in Petrie dishes, and ascitic fluid bouillon in tubes. If desired the bacteria washed off in the different washings are allowed to develop.

Practical Notes on the Examination for Mixed Infection.—1. The difficulties to be overcome, in order to obtain sputum consisting pre-

sumably of exudate from the deeper portions of the lungs, are so great that the collection of the specimens should be supervised by the bacteriologist in charge of the work of examination.

2. Specimens of sputum collected even with the greatest precaution may give evidence of decided mouth infection unless immediately washed.

3. The sputum must be examined very soon after collection.

4. The culture medium used for the final cultures must be suitable for the growth of the micro-organisms.

5. At least two successive examinations of sputum should be made in each case.

6. The results, especially as to the number of colonies, vary according to the size and tenacity of the ball of sputum washed—*e. g.*, a small ball of sputum which becomes more or less broken up upon thorough shaking may contain very few or no bacteria.

Williams, in the examination of the sputum in some 40 cases, came to the following conclusions: 1. The presence of a large number of bacteria in a satisfactory and thoroughly washed specimen of sputum indicates that these bacteria probably play an active part in the disease.

2. The presence of a small number of bacteria in such sputum does not necessarily indicate that they are not active in that case, for they may penetrate more or less deeply into the lung tissue, and produce pathological changes without being thrown off in large numbers with the exudate. It is probable, however, that, as a rule, the smaller the number found the less the degree of mixed infection.

3. Cases of clinically secondary infection frequently give pure cultures of some one organism, which appear to be capable of causing the symptoms.

4. In the majority of severe cases of clinically mixed infection many organisms have been found which usually have belonged to several different species or varieties.

5. In the majority of cases of clinically non-mixed infection very few organisms have been found.

6. Only bacteria which might cause pathological changes were present.

7. Very few of the organisms found were very virulent in rabbits, even though coming from severe cases of mixed infection.

The virulence for laboratory animals of bacteria obtained from the sputum is, therefore, no indication of their virulence for man, because of the impossibility of reproducing in such animals the exact condition of susceptibility present in human infection.

General Rules in Microscopic Examination of Sputum.—Always make two still preparations from each specimen. Report no result as negative until at least two preparations have been subjected to a thorough search with a 1/12 oil-immersion or 2 mm. apochromatic lens by means of a mechanical stage. From a very large experience in the examination of sputum for tubercle bacilli, the New York Health Department bacteriologists have concluded that the examination of

two preparations of each specimen, in the careful manner described above, is usually sufficient to demonstrate the presence of the bacilli when they are present in the sputa, and they are usually found to be present to this extent in fairly well-developed cases of pulmonary tuberculosis, and in many cases which are in the incipient stage. There are, however, undoubted cases of incipient pulmonary tuberculosis which require the examination of many preparations before the tubercle bacillus can be found; and cases also occur in which the sputum for a time does not contain the bacilli, which were, nevertheless, present at an earlier period, and which again later appear. Therefore, if cases occur which may be still regarded as possibly tuberculosis, further examinations of the sputum should be made. It should also be constantly borne in mind that the demonstration of the presence of tubercle bacilli in the sputum proves about as conclusively as anything can the existence of some degree of tuberculosis; but that the absence of tubercle bacilli or the failure to find them microscopically does not positively exclude the existence of the disease. Here tuberculin can be made use of.

Staining of Tubercle Bacilli in Tissues.—Thin sections of tuberculous tissues may be stained by the same methods recommended for cover-glass preparations, except that it is best not to employ heat to any extent. Fixation in bichloride of mercury is better than in alcohol. Formalin is a bad fixative, as it makes the tissues hold the fuchsin with as much tenacity as the bacilli. Both paraffin and celloidin may be used for embedding, but the former is better.

EHRLICH'S METHOD.—Place the paraffin sections in aniline fuchsin and leave at 37° C. for from six to twelve hours, or at about 80° C. for three to five minutes, the sections are then washed in water; then decolorize by placing them for about half a minute in dilute nitric acid (10 per cent.), or in 3 per cent. hydrochloric acid in alcohol; wash in 60 per cent. alcohol until no more color is given off; counterstain for two or three minutes in a saturated aqueous solution of methylene blue, or, better, with hæmatoxylin; wash in water; dehydrate with absolute alcohol; clear in oil of cedar or xylol, and mount in xylol balsam.

METHOD OF ZIEHL-NEELSON.—Stain the section in warmed carbol-fuchsin solution for one hour; the temperature to be not over 45° to 50° C. Decolorize for a few seconds in 5 per cent. sulphuric acid, then in 70 per cent. alcohol, and from this on as in the Ehrlich method.

Inoculation of Animals.—The inoculation of suspected material into guinea-pigs sometimes produces tuberculosis when no bacilli could be detected by microscopic examination. The material may be injected into the subcutaneous tissues, into the peritoneal cavity, or into the mammary gland of a pregnant guinea-pig.

Cultivation.—This is so difficult and requires so much time that it is not used except in important investigations upon the nature of the tubercle bacilli.

CHAPTER XXII.

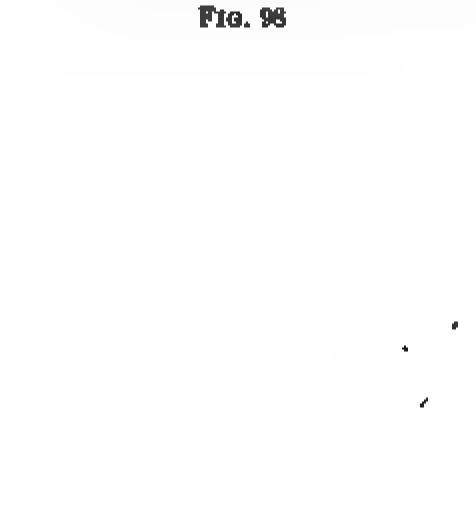
BACILLI SHOWING STAINING REACTIONS SIMILAR TO THOSE OF THE TUBERCLE BACILLI—LUSTGARTEN'S BACILLUS—SMEGMA BACILLUS—LEPROSY BACILLUS—GRASS BACILLI.

Lustgarten's Bacillus—Smegma Bacillus.

Bacilli were discovered by Lustgarten in syphilitic lesions of syphilitic ulcers (1884), and believed by him to be the specific cause of this disease. It has since been shown that in normal smegma from the prepuce or the vulva bacilli are found in great abundance, similar in their morphology to the bacillus of Lustgarten, but differing, as a rule, slightly in certain staining peculiarities. (See Fig. 98.)

Morphology.—Straight or curved bacilli, which bear considerable resemblance to tubercle bacilli, but differ from them in staining reactions. The bacilli are not usually found free in the tissues, but commonly lie singly or sometimes in groups within the interior of cells, having a round, oval, or polygonal form, and apparently somewhat swollen.

Fig. 98



Smegma bacilli, similar in characteristics to Lustgarten's bacillus. × 1100 diameters.

Staining.—The bacillus of Lustgarten *stains* with almost as much difficulty as the tubercle bacillus, but is much less resistant to the action of certain decolorizing agents, such as mineral acids, particularly sulphuric acid.

Biological and Pathogenic Properties.—Numerous attempts have been made to cultivate the bacillus of Lustgarten on artificial media, but with doubtful success. The inoculation of animals has also given only negative results.

Lustgarten's bacillus has been found in various syphilitic tissues and lesions, in beginning sclerosis, in the papules, in condylomata and gummata, and not only in the vicinity of the genitals, but also in the mouth, throat, heart, and brain. No satisfactory experimental evidence has been given of its causative relation to syphilis. The finding of saprophytic bacilli—the so-called smegma bacilli—(Fig. 98 and Plate I., Fig. 4) almost identical morphologically with the bacillus of Lustgarten, under the prepuce of healthy persons, does not prove the identity of the two bacilli, though, in the absence of cultures and inoculation experiments, we have not the means of establishing their relationship to one another. The smegma bacilli have never been identified in other parts

of the body, except in the neighborhood of the genitals. While the bacillus of Lustgarten cannot resist the prolonged decolorizing action of acids, but is resistant to the action of alcohol, the smegma bacillus, when stained, is quickly decolorized by alcohol, but quite resistant to 5 per cent. sulphuric acid solution. Besides, the syphilis bacillus has been found in papules, in gummata, and other syphilomata, where there seems no probability whatever of the smegma bacillus having emigrated. Finally, other bacilli have been described and claimed to be the specific cause of syphilis, but none of these discoveries have been confirmed. The latest micro-organism is discussed under protozoa.

Syphilitic Infection.—Infection of those not immune can take place at any time when an abrasion, however small, is brought in contact with the blood or secretions from the primary or secondary lesions of syphilitics.

The *differential diagnosis* of Lustgarten's bacillus must be made from the tubercle bacillus, the smegma bacillus, and the leprosy bacillus. According to Hueppe, the differential diagnosis between these four organisms depends upon the following reactions: When stained by the carbol-fuchsin method, commonly employed in staining the tubercle bacillus, the syphilis bacillus becomes almost instantly decolorized by treatment with mineral acids, particularly sulphuric acid; whereas, the smegma bacillus resists such treatment for a much longer time, and the lepra and tubercle bacillus for a still longer time. On the other hand, if decolorization is practised with alcohol instead of acids, the smegma bacillus is the first to lose its color. The bacillus tuberculosis and the bacillus of leprosy are both very retentive of their color, even after treatment with acids and alcohol. If, then, we treat the preparation, stained with carbol fuchsin, with sulphuric acid, the syphilis bacillus becomes almost at once decolorized. If it is not immediately decolorized, treat with alcohol; if it is then decolorized, it is the smegma bacillus. If it is still not decolorized, it is either the leprosy or the tubercle bacillus.

By these methods the differential diagnosis can usually be made. In all investigations of importance, however, animal inoculations should also be made, as by this means alone can a positive diagnosis from tuberculosis be established. Especial care should be observed in the examination of syphilitic ulcers of the genital region, as in this situation the smegma bacilli are almost always present.

Leprosy Bacillus.

The bacillus of leprosy was discovered by Hansen and Neisser (1879) in the leprous tubercles of persons afflicted with the disease. This discovery was confirmed by many subsequent observers.

Morphology.—Small, slender rods resembling the tubercle bacilli in form, but somewhat shorter and not so frequently curved. The rods have pointed ends, and in stained preparations unstained spaces, similar to those observed in the tubercle bacillus, are seen. They *stain* readily with the aniline colors and also by Gram's method. Although

differing slightly from the tubercle bacillus in the ease with which they take up the ordinary aniline dyes, they behave like tubercle bacilli in retaining their color when subsequently treated with strong solutions of the mineral acids and alcohol. The slight difference in staining characteristics is too little to be relied upon for diagnostic purposes.

Biological Characters.—Attempts to cultivate the bacillus lepræ have been frequently made, but so far with negative results.

Pathogenesis.—Numerous inoculation experiments have been made on animals with portions of leprous tubercles, but there is no conclusive evidence that leprosy can be transmitted to the lower animals by inoculation. The inference that this bacillus bears an etiological relation to the disease with which it is associated is based entirely upon the demonstration of its constant presence in leprous tissues (Fig. 99).

FIG. 99

Leprosy bacilli in nodule. (Kolle and Wassermann.)

The bacilli are found in all the diseased parts, and usually in large numbers, especially in tubercles on the skin, in the conjunctiva and cornea, the mucous membranes of the mouth, gums, and larynx, and in the interstitial processes of the nerves, testicles, spleen, liver, and kidneys. The rods lie almost exclusively within the peculiar round or oval cells of the granulation tissue which composes the leprous tubercles, either irregularly scattered or arranged parallel to one another. In old centres of infection the leprosy cells containing the bacilli are larger and often polynuclear. Giant cells, such as are found in tuberculosis, are claimed to have been observed by a few investigators (Boinet and Borrel). In the interior of the skin tubercles, the hair follicles, sebaceous and sweat-glands are often attacked, and bacilli have sometimes been found in these (Unna, etc.). Quite young eruptions often contain a few bacilli. A true caseation of the tubercles does not occur, but ulceration results.

In the anæsthetic forms of leprosy the bacilli are found most commonly in the nerves and less frequently in the skin. They have been demon-

strated in the sympathetic nervous system, in the spinal cord, and in the brain. The bacillus lepræ occurs also in the blood, partly free and partly within the leukocytes, especially during the febrile stage which precedes the breaking out of fresh tubercles (Walters and Doutrelepont). The bacilli have also been found in the intestines, in the lungs, and in the sputum, but not in the urine.

With regard to the question of the direct inheritance of the disease from the mother to the unborn child there is considerable difference of opinion. Some cases have been reported, however, in which a direct transmission of the bacillus during intrauterine life seems to be the only or most plausible explanation of the infection. At the same time, we have no positive experimental evidence to prove that such an infection does take place. Although many attempts have been made to infect healthy individuals with material containing the bacilli of leprosy, the results are not conclusive. Even the experiments made by Arning, who successfully infected a condemned criminal in the Sandwich Islands with fresh leprous tubercles, and which has been regarded as positive evidence of the transmissibility of the disease in this way, is by no means conclusive; for, according to Swift, the man had other opportunities for becoming infected. These negative results, together with the fact that infection does not more frequently occur in persons exposed to the disease, may possibly be explained by the assumption that the bacilli contained in the tuberculous tissue are mostly dead, or much more probably that an individual susceptibility to the disease is requisite for its production.

The widespread idea, before the discovery of the leprosy bacillus, that the disease was associated with the constant eating of dried fish or a certain kind of food, has now been entirely abandoned.

The relation of leprosy to tuberculosis is sufficiently evident from their great similarity in many respects. This is rendered still more remarkable if the observation recently made is true, that leprosy reacts, both locally and generally, to an injection of tuberculin in the same manner as tuberculosis (Babes and Kalindero).

Differential Diagnosis.—The differential diagnosis between leprosy and tuberculosis is not difficult in typical cases. The large numbers of bacilli found in the interior of the cells would point with great probability to leprosy. Too much importance should not be placed upon the staining peculiarities, as these are not constant. Moreover, the two diseases not infrequently occur together in the same individual. In making the diagnosis, therefore, all the signs, histological and pathogenic, must be considered and animal inoculations made.

Timothy and Other Grass Bacilli.

On various grasses, in cows' manure, in butter, and in milk, there have been discovered a number of varieties of bacteria which have more or less of the characteristics of the tubercle bacillus. Some of them are as difficult to stain and as resistant to the decolorizing action of

mineral acids and alcohol as the tubercle bacillus found in man. Many of them are of the same general size and shape as the tubercle bacillus, and, strangely enough, produce in animals small diseased areas which not only macroscopically but also microscopically resemble miliary tubercles due to the tubercle bacillus. They are, however, entirely different in their culture characteristics, producing in twenty-four to forty-eight hours, on ordinary culture media, moist, round colonies of an eighth to a quarter of an inch in diameter, and of a more or less intense pink color. In animals they produce only localized lesions, causing death only when injected in large numbers. The injected animals are unaffected by tuberculin injections. The chief interest which these bacilli have for us is the possibility of confusing them with the tubercle bacilli. This danger is always present in milk, for the grass bacilli find so many means of gaining entrance to it. In the examination of dust, healthy throat and nose secretions, etc., the simple microscopic examination might lead to error.

They can be separated from tubercle bacilli by inoculating animals in which no progressive lesions will develop. If there is any doubt about the nature of the infection, inject $\frac{1}{4}$ c.c. of tuberculin, when if infected with tuberculosis they will die, but if by grass bacilli they will show no reaction. Cultures from the lesions will also show, on ordinary media, pink colonies if grass bacilli are present, and no growth if only tubercle bacilli.

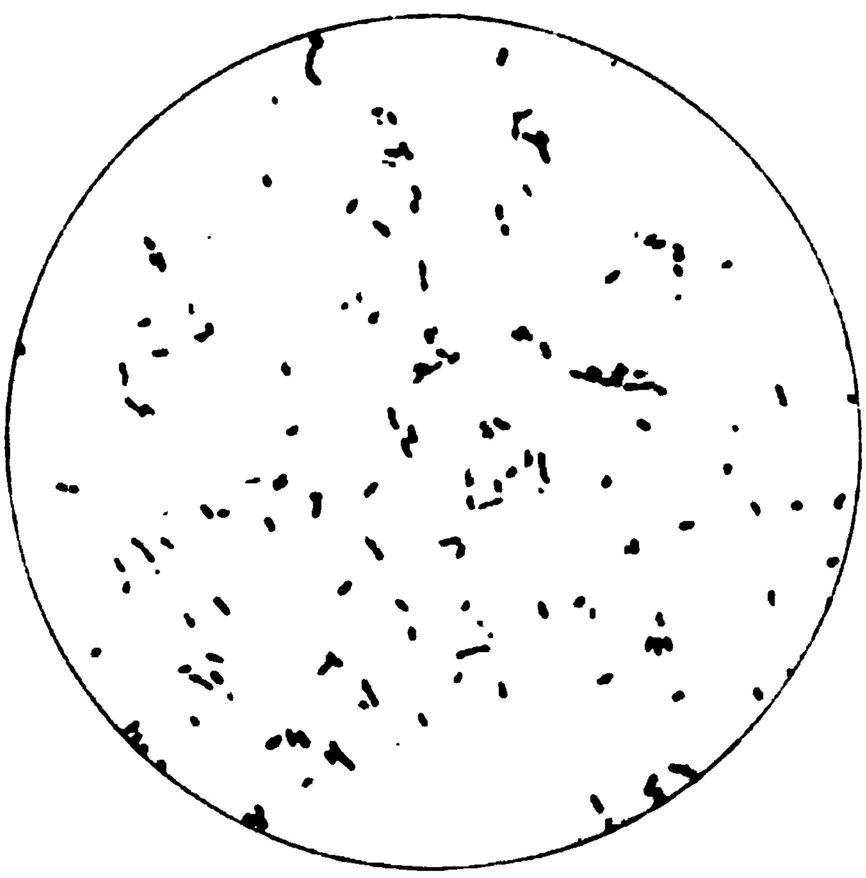
CHAPTER XXIII.

THE INFLUENZA AND PSEUDOINFLUENZA BACILLI—THE KOCH-WEEKS BACILLUS.

The Influenza Bacillus.—Influenza as a distinct entity can be traced back to the fifteenth century and probably existed at a much earlier date.

At times but few endemic cases occur and then a great epidemic spreads over the civilized world. The last great epidemic reached Russia from the East in the fall of 1889 and gradually spread over Europe and to America, reaching the latter country in December of that year. Since then we have had more or less of it, especially during the winter months.

FIG. 100



Influenza bacilli. × 1100 diameters.

The rapidity of the spread of epidemics of influenza suggested that persons were the carriers of the infection, while the location of the disease pointed to the respiratory tract as the location of and to the expectoration as the chief source of infection by the micro-organisms.

After numerous unsuccessful attempts, during the epidemic of 1889 and succeeding years, to discover the specific cause of influenza, Pfeiffer (1892) succeeded in isolating and growing upon blood agar a bacillus which abounded in the purulent bronchial secretion of patients suffering from epidemic influenza, which he showed was the probable cause of the disease. This bacillus was not found upon normal respiratory mucous membranes.

Morphology.—Very small, moderately thick bacilli (0.2 to 0.3μ in thickness to 0.5 to 1.5μ in length), usually occurring singly or united in pairs, but threads or chains of three, four, or more elements, are occasionally found. No capsule has been demonstrated.

Staining.—The bacillus *stains* with difficulty with the ordinary aniline colors—best with dilute Ziehl's solution (water 9 parts to Ziehl's solution 1 part), or Loeffler's methylene-blue solution, with heat. When faintly stained the two ends of the bacilli are sometimes more deeply stained than the middle portion. They are not stained by Gram's method.

Biology.—An aërobic, non-motile bacillus; does not form spores; no growth occurs with most cultures below 26° C., or above 34° C., or in the entire absence of oxygen.

Cultivation.—This bacillus is best cultivated at 37° C., and on the surface of ordinary nutrient culture media containing hæmoglobin. Plain or *glycerin agar*, or *blood serum* thinly streaked with rabbit or human blood, make the best media for their growth. At the end of eighteen hours in the incubator very small circular colonies are developed, which, under a low magnification (100 diameters), appear as shining, transparent, homogeneous masses, and even under a No. 7 lens scarcely show at all the individual organisms. Older colonies are sometimes colored yellowish-brown in the centre. A characteristic feature of the influenza bacillus is that the colonies tend to remain separate from each other, although when they are thickly sown in a film of moist blood upon nutrient agar they may become confluent. Transplantation of the original culture to ordinary agar or serum cannot, as a rule, be successfully performed, owing to the want of sufficient hæmoglobin; but if sterile rabbit, pigeon, or human blood be added to these media transplantation may be indefinitely performed, provided it is done every three or four days. Cultures may remain alive up to seventeen days in the ice-chest. By a series of beautifully carried out experiments Pfeiffer showed that not only were the red blood cells the necessary part of the blood needed for the growth of the influenza bacillus, but that it was the hæmoglobin in the cells.

In *bouillon* in thin layers, to which blood is added, a good development takes place if there is free access of oxygen.

Resistance and Length of Life.—The influenza bacillus is very sensitive to desiccation; a pure culture diluted with water and dried is destroyed with certainty in twenty-four hours; in dried sputum the vitality, according to the completeness of drying, is retained from twelve to forty-eight hours. It does not grow, but soon dies in water. The thermal death point is 60° C. with five minutes' exposure (Pfeiffer and Beck). In blood-bouillon cultures at 20° C. they retain their vitality for from a few days to two or three weeks. In moist sputum it is difficult to determine the duration of their life, since the other bacteria overgrow and make it impossible to find them. It is probable that they can remain alive for at least two weeks. The bacilli are very readily killed by chemicals, disinfectants, and succumb to boiling within one minute and to 60° C. within ten minutes.

Detection of the Influenza Bacillus in Sputum.—The direct microscopic examination of stained smears of sputum may give considerable information as to the probable presence of influenza-like bacilli. The frequent presence of other influenza-like bacilli in the throat secretions leads to so much doubt that it is advisable from the start to make use of plate cultures, the best medium being nutrient agar freshly smeared with a film of rabbit's blood.

Pathogenesis.—The bacillus of influenza, in so far as experiments show, produces a disease at all similar to influenza only in monkeys and to a less extent in rabbits. When a small quantity of culture on blood agar, twenty-four hours old, suspended in 1 c.c. of bouillon, was injected intravenously into rabbits, Pfeiffer found that a characteristic pathogenic effect was produced. The first symptoms were developed in one and a half to two hours after the injection. The animals became extremely feeble, lying flat upon the floor, with their limbs extended, and suffered from extreme dyspnœa. The temperature rose to 41° C. or above. At the end of five or six hours they were able to sit up on their haunches again, and in twenty-four hours had recovered. Larger doses caused the death of the animals inoculated. These results are attributed by Pfeiffer to toxic products present in the cultures, and in none of his experiments was he ever able to obtain effects resembling septicæmic infection. In some of the experiments on monkeys, these animals, when cultures were rubbed into the nasal mucous membrane, showed a febrile condition, lasting for a few days; but in no instance has Pfeiffer observed a multiplication of the bacilli introduced.

The cell bodies of the bacilli seem to possess considerable pyogenic action.

Immunity.—Possibly an immunity for a short period against the influenza poison may be established after an attack. At least in three experiments made by Pfeiffer on monkeys, these animals, after recovering from an inoculation with bacilli, seemed to be much less susceptible to a second injection.

Distribution of Influenza Bacilli in the Body.—In patients suffering from influenza the bacilli are found chiefly in the nasal and bronchial secretions. In acute uncomplicated cases they may be observed microscopically in large masses, and often in absolutely pure culture; the green, purulent sputum derived from the bronchial tubes is especially suitable for examination. The older the process is, the fewer free bacilli will be found and the more frequently will they be seen lying within the pus cells, instead of being embedded free in the secretion as at first. At the same time they stain less readily and present more irregular and swollen forms. Very frequently the influenza process invades portions of the lung tissue. In severe cases a form of pneumonia is the result, which is lobular and purulent in character, and accompanied by symptoms which may be somewhat characteristic for influenza, or, again, almost identical with bronchopneumonia due to the pneumococcus. The walls of the bronchioles and alveolar septa become densely infiltrated with leukocytes, and the spaces of the bronchial tubes and

alveoli become filled. The influenza bacilli are found crowded in between the epithelial and pus cells and also penetrate the latter. There may be partial softening of the tissues, or even abscess formation. Bacilli are found in fatal cases to have penetrated from the bronchial tubes not only into the peribronchitic tissue, but even to the surface of the pleura, and rarely they have been obtained in pure cultures in the pleuritic exudation. The pleurisy which follows influenza, however, is usually a secondary infection, due to the streptococcus or pneumococcus.

Presence of Influenza Bacilli in Chronic Influenza and in Tuberculosis.—Ordinarily influenza runs an acute or subacute course, and not infrequently it is accompanied by mixed infections with the pneumococcus and the streptococcus. Pfeiffer was the first to draw attention to certain chronic conditions depending upon the influenza bacillus. Bacilli may be retained in the lung tissue for months at a time, remaining latent a while, and then becoming active again, with a resulting exacerbation of the disease. Consumptives are liable to carry influenza bacilli for years and are particularly susceptible to attacks of influenza. Williams, in the examination of sputa in cases of pulmonary tuberculosis, has found abundant influenza bacilli to be present in a large proportion of the samples of sputum from consumptives, and this not only in winter but also in the summer, when no influenza was known to be present in New York. Taken together with results elsewhere, this indicates that at all times of the year many consumptives carry about with them influenza bacilli, and that very likely many healthy persons as well as persons suffering from bronchitis also harbor a few. Given proper climatic conditions, we have at all times the seed to start an epidemic.

The influenza bacillus does not occur, as a rule, in the blood and probably does not develop there. It is found at times in otitis media accompanying influenza, and has been found in the meninges in cases of meningitis. So far as positive results have shown, influenza would seem to be almost always a local infection confined chiefly to the air passages. The general, cerebral, gastric, and other symptoms produced are due to the absorption of the toxic products of the specific organism, these poisons being particularly active in their effects on the central nervous system.

The discovery of this bacillus enables us to explain many things, previously unaccountable, in the cause of epidemic influenza. We now know, from the inability of the influenza bacillus to exist for long periods in dust, that the disease is not transmissible to great distances through the air. We also know that the infective material is contained only in the catarrhal secretions. Sporadic cases, or the sudden eruption of epidemics in any localities from which the disease has been absent for a long time, or where there has been no new importation of infection, may possibly be explained by assuming that the bacilli, as already mentioned, often remain latent in the lungs or bronchial secretions of the body for many months. and perhaps years, and then

become active again, when under favorable circumstances they may be communicated to others. The bacteriological diagnosis of influenza is of considerable importance for the identification of clinically doubtful cases, which, from their symptoms, may be mistaken for grippe, or *vice versa*. Among these are bronchitis, pneumonia, or tuberculosis. Up to the present time the diagnosis gives us little help in treatment.

In acute uncomplicated cases the probable diagnosis can be frequently made by microscopic examinations of stained preparations of the sputum, there being present enormous numbers of small bacilli, at first largely free and later largely in the pus cells. In chronic cases or those of mixed infection few or many bacilli may be found and the culture method may be necessary to give even a probable diagnosis. The bacillus of influenza is not readily separated by its morphological, staining, and cultural peculiarities from other bacteria belonging to the influenza group and at present it is almost impossible to certainly identify it.

The Pseudoinfluenza Bacillus.—This bacillus is culturally very similar to the typical influenza bacillus, but may be distinguished from it by its larger size and tendency to grow out into long threads. It is not certain but that it is a form of the influenza bacillus.

Whooping-cough Bacillus.—In this disease bacilli are regularly found which differ but slightly from the influenza bacilli. They produce, when injected in animals, agglutinins which are specific for them, but not for influenza bacilli. Both bacilli are affected by the same group (Wollstein). The blood of those suffering from whooping-cough agglutinates the whooping-cough bacilli but not the influenza bacilli. Further investigation is required to establish whether they are the exciting factor of the disease. The Koch-Weeks bacillus is also very similar to the influenza bacillus.

Relation of the Clinical Symptoms to the Bacterial Excitant.—There is no doubt that other infections are also included under the clinical forms of influenza, and during an epidemic of bronchopneumonias, irregular types of lobar pneumonias, and cases of bronchitis frequently have symptoms so closely alike that the nature of the bacteria active in the case is very frequently different from that supposed by the clinician. Thus in four consecutive autopsies examined by the writer the influenza bacillus was found almost in pure culture in one case believed to be due to the pneumococcus, and entirely absent in two of the three believed to be due to it. Except for these examinations the clinician would be of the opinion that he had clearly diagnosed bacteriologically the cases, while in fact he had been wrong in three of the four.

The striking symptoms in acute respiratory diseases are frequently more due to the location of the lesions than to the special variety of organisms producing them. In epidemics of influenza there are, of course, many cases which, on account of their characteristic symptoms, can be fairly certainly attributed to the influenza bacilli. Even under these circumstances error may be made, as, for instance, two cases of apparently typical influenza were reported in a household and both

showed a total absence of influenza bacilli. The pneumococcus was present in almost pure culture.

Examination of Sputum for Influenza Bacilli.—1. Sputum coughed from the deeper air passages and not from throat scraping should be used.

2. The sputum should be received in a sterile bottle, which should then be placed immediately in cracked ice.

3. Blood-agar plates should be made by dropping a drop of fresh rabbit's blood, obtained aseptically, on the centre of a hardened agar plate.

4. One of the more solid masses of the sputum should be taken from the bottle with sterile forceps and placed on a plain agar plate. A small portion of this mass should be separated with a sterile platinum needle and drawn through the blood on the blood-agar plate from the centre out in different directions. The larger part of what is left of this small portion is then placed in a similar manner over a second blood agar, and from this to a third, sterilizing the needle between the transfers. The plates should be placed in the thermostat for twenty-four hours.

5. After the plates are planted two smears should be made, one stained by Gram and the other by weak carbol-fuchsin.

6. After twenty-four hours the plates are examined under low power. The influenza colonies use up the hæmoglobin, and in parts of the blood-agar plate where the blood is of right thickness such colonies show as almost clear areas surrounded by the red blood. With a higher power (No. 6 or 7 objective), if such areas seem to be made up of fine indefinite granulations, they are practically sure to be influenza colonies. Most influenza colonies are more highly refractive than other light colonies, and they show this characteristic best when they grow on the edge of a blood mass. Many influenza colonies also show heapings in the centre. Influenza colonies growing away from the blood cells are less characteristic in appearance and less easily differentiated from other bacteria.

7. Fishings from the influenza-like colonies should be planted on blood-agar tubes, and if, after twenty-four hours in the thermostat, the resulting growth should consist of influenza-like organisms, plantings should be made on plain agar. The first generation on plain agar may show slight growth because of the blood carried over from the original tube, but the second generation should show no growth if the organism is the influenza bacillus.

8. The agglutination characteristics of the cultures should be tested in the serum from a rabbit injected with a single typical culture, and in the serum from one injected with a number of cultures. The agglutination tests should be carried out in order to gain information. The cultures tested in the Research Laboratory have shown considerable variation.

For Testing the Agglutination of Influenza Bacilli in the Hanging Drop.—Grow the cultures on slanted agar tubes to which, after cooling to 40° C., ½ c.c. of defibrinated blood has been added. When twenty to

twenty-four hours old make a suspension of the bacilli in normal salt solution, controlling the number of bacilli by examining a hanging-drop preparation. The influenza bacilli seem to agglutinate rather slowly; so it usually takes four to five hours to get a good reaction.

Serum Therapeutics.—No protective serum has been produced which has any value in treatment.

The Koch-Weeks Bacillus of Conjunctivitis.

This bacillus was first observed by R. Koch in 1883 while making certain investigations into inflammation of the eye occurring during an epidemic of cholera in Alexandria. It was later, in 1887, more specifically described by Weeks in New York. Weeks obtained it in pure culture in 1890.

The infective disease which is caused by this bacillus seems to be very widely distributed, no land or clime probably being exempt from it. In this country it occurs epidemically and with increasing frequency during the Spring and Fall months. Weeks has found the bacillus in over 1000 cases in New York City.

Morphology.—The bacilli from the purulent secretions are small and slender, being not unlike the influenza bacilli but somewhat longer. They vary in length from 0.5 to 1μ or even 2μ occasionally; the longer forms are apparently unions of thread-like filaments. The shorter bacilli not infrequently have the appearance of diplococci. Sometimes they exhibit slight polar staining. Their width is very constant. The ends are rounded. They are rapidly decolorized by Gram.

Staining.—They are best stained by very dilute solutions of carbol-fuchsin or Loeffler's methylene blue, but do not stain readily.

In smear preparations the Koch-Weeks bacilli are, as a rule, seen alone or associated with isolated cocci and bacilli, especially xerosis bacilli. They are not infrequently observed within the cells, and are very rarely associated with gonococci and pneumococci, such mixed infections being extremely uncommon.

Biological Characters.—The Koch-Weeks bacillus grows only at incubator temperature. Of the ordinary culture media none but moist and slightly alkaline peptone agar can be employed. Special media are required generally for its cultivation. The best results have been obtained with serum agar or a mixture of glycerin agar and ascitic fluid, 2 to 1. Pure cultures are rarely obtained at first; they are usually associated with colonies of xerosis bacilli or staphylococci. After twenty-four to forty-eight hours the colonies are noticeable as moist, transparent, shining drops or points. Microscopically examined under low magnifying power they appear like small gas bubbles; by closer examination they are seen to be round, lying loosely on the surface, and are readily removed. Under higher magnification a number of fine points are observable. The colonies, which resemble those of influenza, have a tendency to confluesce, but are not so sharply defined

as the latter and become more quickly indistinguishable. Isolated colonies, especially those in the neighborhood of xerosis bacilli or staphylococci, grow larger and their contour is slightly wavy; they are more opaque and granular than influenza colonies. In serum or blood bouillon a slight cloudiness is produced which finally settles down.

Resistance.—In culture media the bacilli die rapidly, seldom living more than five days. Development ceases at 20°. They resist a temperature of 50° for ten minutes, but cannot withstand 60° for more than one or two minutes. Kept for one and a half hours at —7° they still remain alive. Exposure to the sun's rays for one-half hour does not kill them, but at the end of two and one-half hours they die. They cannot resist dying for any length of time.

Transmission.—This occurs only by contact either by direct or indirect conveyance of the moist infective material. Infection is not communicated through the air by means of dust, as the bacilli soon die when dried. It may, however, be conveyed by flies, etc.

Pathogenesis.—The Koch-Weeks bacillus is not pathogenic for animals. Man, on the contrary, is extremely susceptible to infection from this bacillus, which produces one of the most contagious diseases known.

Immunity is not produced to any extent by one attack, but there does seem to be an individual susceptibility to the disease.

Differential Diagnosis.—The only micro-organisms from which the Koch-Weeks bacillus would seem to require differentiation are the influenza bacillus of Pfeiffer, the so-called influenza bacillus of conjunctivitis of Müller and the pseudoinfluenza bacillus of Zur Nedden. These latter bacilli, however, grow well only on hæmoglobin media, which the Koch-Weeks bacillus does not require. The colonies on serum agar are smaller than those of the influenza bacilli and the edges are more granular. While the influenza bacillus is slightly pathogenic for certain animals, the Koch-Weeks bacillus has so far given negative results with all animals. Clinically also the disease is distinctly different.

CHAPTER XXIV.

THE PRODUCERS OF ABSCESSES, CELLULITIS, SEPTICÆMIA, ETC.

THE STAPHYLOCOCCI.

STAPHYLOCOCCI were first obtained from pus by Pasteur in 1880. In 1881 Ogston showed that they frequently occurred in abscesses, and in 1884 Rosenbach fully demonstrated their etiological importance in circumscribed abscesses, osteomyelitis, etc. Of all the staphylococcus varieties the staphylococcus pyogenes aureus is by far the most important and will, therefore, be first described.

The Staphylococcus Pyogenes Aureus.

The staphylococcus pyogenes aureus is one of the commonest pathogenic bacteria, being usually present in the skin or mucous membranes, and is the organism most frequently concerned in the production of acute, circumscribed, suppurative inflammations.

Morphology.—Small, spherical cells, having a diameter of 0.7μ to 0.9μ, occurring solitary, in pairs as diplococci, in short rows of three or four elements, or in groups of four, but most commonly in irregular masses, simulating clusters of grapes; hence the name *staphylococcus*. (See Fig. 101.)

Staining.—It *stains* quickly in aqueous solutions of the basic aniline colors and with many other dyes. When previously stained with gentian violet it is not decolorized by Gram's method. When slightly stained each sphere frequently is seen to be already dividing into two semispherical bodies.

Biology.—The staphylococcus pyogenes aureus is an aërobic, *facultative anaërobic* micrococcus, growing at a temperature from 8° to 43° C., but best at 25° to 35° C. The staphylococci grow readily on all the common laboratory media, such as milk, bouillon, nutrient gelatin, or agar. A slightly alkaline reaction to litmus is best for the growth of the staphylococci, but they also grow in slightly acid media.

Cultivation. GROWTH IN NUTRIENT BOUILLON.—The growth of the staphylococcus is rapid, reaching about 50,000,000 per c.c. at the end of twenty-four hours at 30° C. The bouillon is cloudy and frequently has a thin pellicle. Later a shiny sediment forms. The odor is disagreeable. In peptone-water growth occurs with indol production.

GROWTH ON GELATIN.—Grown on gelatin plates it develops, at room-temperature, within forty-eight hours, punctiform colonies, which, when examined under a low-power lens, appear as circular disks of a pale-

brown color, somewhat darker in the centre, and surrounded by a smooth border. The colonies grow rapidly. The appearance of the growth is most characteristic. Immediately surrounding the colonies, which are of a pale-yellow color, there is a deepening of the surface of the gelatin, due to its liquefaction. By suitable light a number of these shallow depressions with sharply defined outlines may be seen on the gelatin plate, having a diameter of from 5 to 10 mm., in the centres of which lie the yellow colonies. Later, the liquefaction becomes general, the colonies running together. In stab cultures in gelatin a white confluent growth at first appears along the line of puncture, followed by liquefaction of the medium, which rapidly extends to the sides of the test-tube. At the end of two days the yellow pigmentation begins to form, and this increases in intensity for eight days. Finally, the gelatin is completely liquefied, and the staphylococci form a golden-yellow or orange-colored deposit at the bottom of the tube. Under unfavorable conditions the staphylococcus aureus gradually loses its ability to make pigment and to liquefy gelatin.

Fig. 101

Staphylococcus. × 1100 diameters.

Growth on Agar.—In streak and stab cultures on agar a whitish growth is at first produced, and this at the end of a few days becomes a faint to a rich golden-yellow on the surface. The yellow pigmentation is produced only in the presence of oxygen; colonies found at the bottom of a stab culture or under a layer of oil remain white.

Milk.—Milk inoculated with this micrococcus is coagulated at the end of from one to eight days.

Growth on Potato.—Upon this substance the staphylococci grow readily and produce abundant pigment.

Growth on Loeffler's Solidified Blood Serum.—Growth vigorous, with fairly good pigment production. Some varieties slowly liquefy the serum.

Growth on Blood Agar.—If nutrient agar to which a little animal blood has been added is streaked with staphylococci there appears, at the end of twenty-four hours at 35° C., about the growth a clear zone, owing to the hæmolytic effect of the staphylococcus products.

In certain culture media, as the result of the growth of the staphylococcus aureus, there is a *production of acid* in considerable quantities, these consisting chiefly of lactic, butyric, and valerianic acids. These acids have been supposed to play a part in the production of pus, in which, according to some observers, they are often present.

Resistance.—The staphylococcus is distinguished from most other pathogenic bacteria by its comparatively greater power of resistance to outside influences, desiccation, etc., as well as to chemical disinfectants. Cultures of the staphylococcus pyogenes in gelatin or agar retain their

vitality for a year or more. Suspended in water its thermal death point varies with different cultures and averages about two hours at 50° C., one-half to one hour at 60° C., ten minutes at 70° C., and five minutes at 80° C. Upon silk threads and in media rich in organic matter its resistance is greater, but subjected to 80° C. for thirty minutes or boiling for two minutes it is almost surely killed. Cold has but little effect. Thirty per cent. of the organisms remained alive after being subjected by us to freezing in liquid air for thirty minutes.

They are quite resistant to direct sunlight and to drying. Dried pus contains living staphylococci for weeks and even months, and they can be found alive in the fine dust of the air in living and in operating rooms.

Effect of Chemicals.—In water they remain alive for several weeks. To most disinfectants the staphylococci are rather resistant. The presence with staphylococci of organic substances, especially albumin, increases their resistance. In watery solution dissolved mercuric chloride, 1:1000, destroys the organisms in five to fifteen minutes but when in pus not for several hours.

Hydrogen peroxide in 1 per cent. solution kills in about one-half hour. Methyl alcohol in 50 per cent. solution kills staphylococci on silk threads in ten minutes. The same effect is obtained by carbolic acid in 3 per cent. solution or lysol in 1 per cent. solution. Formaldehyde in watery solution acts only in concentrations of 5 per cent. or over.

Pathogenesis.—The pathogenic effect of the staphylococcus pyogenes aureus on test animals varies considerably, according to the mode of application and the virulence of the special culture employed. In the experiments so far made this micrococcus, as found in suppurative processes in the human subject, has not proved to be as infectious for animals as it is for man. In man a simple rubbing of the surface of the unbroken skin with pus from an acute abscess is, as a rule, sufficient to produce purulent inflammation, and the introduction of a few germs from a septic case into a wound may lead to a fatal pyæmia. These conditions can only be reproduced in lower animals with difficulty, and by the inoculation of large quantities of the culture. Subcutaneous injections, or the inoculation of open wounds in mice, guinea-pigs, and rabbits, are commonly without result; occasionally abscess formation may follow at the point of inoculation, which usually ends in recovery. The pus-producing property of the organism is exhibited in proportion to the virulence of the culture employed. Slightly virulent cultures, which constitute the majority of those obtained from pus taken from the human subject, when injected subcutaneously in large quantities (several c.c. of a fresh bouillon culture) in rabbits or guinea-pigs, give rise to local pathological lesions--acute abscesses. When virulent cultures are used—which are rarely obtainable—0.5 c.c. of a fresh bouillon culture is sufficient to produce similar results. The abscesses generally heal without treatment; sometimes the animals die from marasmus in consequence of the suppurative process. In intraperitoneal inoculations the degree of virulence of the culture employed is still more conspicuous in the effects produced. The animals usually die in

from two to nine days. The most characteristic pathological lesions are found in the kidneys, which contain numerous small collections of pus, and under the microscope present the appearances resulting from embolic nephritis. Punctiform, whitish-yellow masses of the size of a pea are found permeating the pyramids. Many of the capillaries and some of the smaller arteries of the cortex are plugged up with thrombi, consisting of micrococci. Metastatic abscesses may also be observed in the joints and muscles. The micrococci may be recovered in pure cultures from the blood and the various organs; but they are not numerous in the blood and are often difficult to demonstrate microscopically. Intravenous inoculations of animals are followed by similar pathological changes. Orth and Wyssokowitsch first pointed out that injection of staphylococci into the circulation of rabbits whose cardiac valves have previously been injured produced ulcerative endocarditis. Subsequently, Weichselbaum, Prudden, and Fraenkel and Sänger obtained confirmatory results, thus establishing the fact that when the valves are first injured, mechanically or chemically, the injection into a vein of a pure culture of staphylococcus aureus gives rise to a genuine ulcerative endocarditis. It has been further shown by Ribbert that the same result may be obtained without previous injury to the valves by injecting into a vein the staphylococcus from a potato culture suspended in water. In his experiments not only the micrococci from the surface but the superficial layer of the potato were scraped off with a sterilized knife and mixed with distilled water, and the successful result is ascribed to the fact that the little agglomerations of micrococci and infected fragments of potato attach themselves to the margins of the valves more readily than isolated cocci would do. Not infrequently, also, in intravenous inoculations of young animals there occurs a localization of the injected material in the marrow of the small bones. This may take place in full-grown animals when the bones have been injured or fractured. The experimental osteomyelitis thus produced has been demonstrated to be anatomically analogous to this disease in man. With regard to the lesions found in the kidneys after intraperitoneal or intravenous inoculation of cultures of the staphylococcus, it has been found that when injected in considerable quantities the organism may be obtained in cultures from the urine, but not sooner than six or eight hours after the injection, and not until the formation of purulent foci in the kidneys has already occurred.

Toxic Substances.—Filtrates of cultures contain very toxic substances. Injected into the peritoneal cavity they excite peritonitis. Under the skin they produce infiltration or abscess. In the blood they injure both the red and white corpuscles.

Cultures of the staphylococcus, when sterilized by boiling and injected subcutaneously, produce marked positive chemotaxis and often local abscesses. Leber found also that sterilized cultures introduced into the anterior chamber of the rabbit's eye would bring about a fibropurulent inflammation, the cornea becoming infiltrated, and perforation alongside of the sclerotic ring finally taking place. This was followed by the formation of pus in the anterior chamber and recovery. These

local changes follow the inoculation of small quantities only of the dead cultures; but when large amounts are injected into a vein or into the abdominal cavity, toxic effects are produced. Dogs and guinea-pigs thus treated usually die, showing symptoms of poisoning. The hæmolytic effect of certain products of virulent staphylococci have recently been studied. In cultures they can be detected about the third or fourth day and reach their maximum on the ninth to fourteenth day. Virulent staphylococci are more apt to produce this substance than the non-virulent, but there is no definite rule.

A poison which injures leukocytes is also produced. This is destroyed at about 57° C.

Other poisons of less definite properties are also found in the filtrate.

Immunization.—Immunity against staphylococcus infection may be produced in different animal species by the injection of increasing doses of the pure culture, either living or previously sterilized by heating. Wright claims to have injected dead cultures with good results in persons suffering from staphylococcus skin infections.

The blood serum of animals which have been immunized by means of living or dead cultures possesses slight immunizing and curative effects in other animals, but no practical use of the serum has been attempted in man.

Occurrence in Man.—Practically all micro-organisms have been shown by experiment to produce, under certain conditions, the formation of pus by their products when inoculated into the animal body; but, while this has been demonstrated, the researches of bacteriologists show that only a few species are usually concerned in the production of acute abscesses in man. Of these the two most important, by reason of their frequent occurrence and pathogenic power, are staphylococcus pyogenes and streptococcus pyogenes. These two organisms are often found in the same abscess; thus, Passet, in 33 cases of acute abscess, found staphylococcus aureus and albus associated in 11, albus alone in 4, albus and citreus in 2, streptococcus pyogenes alone in 8, albus and streptococcus in 1, and albus, citreus, and streptococcus in 1. The staphylococcus is liable to enter as a mixed infection into most infections due to other bacteria, and is almost always met with in all inflammations of the skin and mucous membranes or in cavities connected with them. Care must always be taken that the simple finding of staphylococci does not cause one to overlook other organisms, which perhaps were the original exciters of the diseased process.

As the result of extended researches the golden staphylococcus has been demonstrated not only in furuncles and carbuncles, but also in various pustular affections of the skin and mucous membranes—impetigo, sycosis, phlyctenular conjunctivitis; in purulent conjunctivitis and inflammation of the lacrymal sac; in acute abscesses formed in the lymphatic glands, the parotid gland, the tonsils, the mammæ, etc.; in metastatic abscesses and purulent collections in the joints; in empyema, infectious osteomyelitis, ulcerative endocarditis, pyelonephritis, abscess of the liver, phlebitis, etc. It is one of the chief etiological factors

in the production of pyæmia in the various pathological forms of that condition of disease. It is remarkable how many staphylococci may be present in the blood without a fatal result, if the original source of infection is removed. We met with one case in which over 800 staphylococci were present in 1 c.c. of blood. A week later only five were found. The patient finally died from pneumonia.

Not all persons are equally susceptible to infection by the staphylococcus; those who are in a cachectic condition or suffering from constitutional diseases, like diabetes, are especially predisposed to infection. In healthy individuals certain parts of the body, as the back of the neck and the buttocks, are more liable to be attacked than others, with the production of furuncles, carbuncles, etc. In persons in whom sores are readily caused, in consequence of disturbances of nutrition, as in exhausting diseases, the micrococci settle at the points of least resistance. Such conditions are present in the bones of debilitated young children, in fractures, and injuries in general.

The pyogenic properties of the staphylococcus have been demonstrated upon man by numerous experiments. Garré inoculated a small wound at the edge of one of his finger-nails with a minute quantity of a pure culture, and purulent inflammation, extending around the margin of the nail, resulted from the inoculation. Staphylococcus aureus was recovered in cultures from the pus thus formed. The same observer applied a considerable quantity of a pure culture obtained from this pus—third generation—to the unbroken skin of his forearm, rubbing it well into the skin. At the end of four days a large carbuncle, surrounded by isolated furuncles, developed at the point where the culture had been applied. This ran the usual course, and it was several weeks in healing. No less than seventeen scars remained to testify to the success of the experiment.

Staphylococcus Pyogenes Albus.

It is morphologically identical with the staphylococcus pyogenes aureus, and is probably the same organism which has lost the property of producing pigment. On the average it is somewhat less pathogenic and seldom produces pyæmia or grave infections. The surface cultures upon nutrient agar and potato have a milk-white color. Its biological characters are not to be distinguished from the staphylococcus aureus.

The majority of bacteriologists agree with Rosenbach, that the aureus is found at least twice as frequently in human pathological processes as the albus.

Staphylococcus Epidermidis Albus (Welch).

Probably identical with the staphylococcus pyogenes albus. With reference to this micrococcus, Welch says: "So far as our observations extend—and already they amount to a large number—this coccus may

be regarded as nearly, if not quite, a constant inhabitant of the epidermis. It is now clear why I have proposed to call it the staphylococcus epidermidis albus. It possesses such feeble pyogenic capacity, as is shown by its behavior in wounds, as well as by experiments on rabbits, that the designation staphylococcus pyogenes albus does not seem appropriate. Still, I am not inclined to insist too much upon this point, as very probably this coccus—which has hitherto been unquestionably identified by others with the ordinary staphylococcus pyogenes albus of Rosenbach—is an attenuated or modified form of the latter organism, although, as already mentioned, it presents some points of difference from the classical description of the white pyogenic coccus."

According to Welch, this coccus differs from the staphylococcus albus in the fact that it liquefies gelatin more slowly, does not so quickly cause coagulation in milk, and is far less virulent when injected into the circulation of rabbits. It has been shown by the experiments of Bossowski and of Welch that this micro-organism is very frequently present in aseptic wounds, and that usually it does not materially interfere with the healing of wounds, although sometimes it appears to cause suppuration along the drainage tube, and it is the common cause of "stitch abscess."

Staphylococcus Pyogenes Citreus and other Staphylococci.

Isolated by Passet (1885) from the pus of acute abscesses, in which it is occasionally found in association with other pyogenic cocci. It is distinguished from the other species only by the formation of a lemon-yellow pigment.

Many other varieties of staphylococci have been occasionally met with which differ in some respects from the typical varieties. This difference may be in the fact that they liquefy gelatin more slowly or not at all, or in pigment formation, or in agglutination, or in still other respects. None of these varieties are of great importance.

The Micrococcus Tetragenus.

This organism was discovered by Gaffky (1881). It is not infrequently present in the saliva of healthy individuals and in the sputum of consumptive patients. In sputum it is sometimes an evidence of mouth contamination rather than lung infection. It has repeatedly been observed in the walls of cavities in pulmonary tuberculosis associated with other pathogenic bacteria, which, though playing no part in the etiology of the original disease, contribute, doubtless, to the progressive destruction of the lung. Its pyogenic character is shown by its occasional occurrence in the pus of acute abscesses. Its presence has also been noted in the pus of empyema following pneumonia.

Morphology.—Micrococci having a diameter of about 1μ, which divide in two directions, forming tetrads, and bound together by a transparent, gelatinous substance, enclosing the cell like a capsule. In cultures the cocci are seen in various stages of division as large, round,

undivided cells, in pairs of oval elements, and in groups of three and four (Fig. 102). When the division is complete they remind one of sarcinæ in appearance, except that they do not divide in three directions and are not built up like diminutive cotton bales.

Staining.—This micrococcus *stains* readily with the ordinary aniline dyes; the transparent gelatinous envelope is only feebly stained. It is not decolorized by Gram's method.

Biology.—The growth of this micrococcus is slow under all conditions. It grows both in the presence and absence of oxygen; it grows best from 35° to 38° C., but may be cultivated also at the ordinary room-temperature—about 20° C.

Growth on Gelatin.—On gelatin plates small, white colonies are developed in from twenty-four to forty-eight hours, which, when examined under a low-power lens, are seen to be spherical or lemon-shaped,

Fig. 102

Micrococcus tetragenus. × 1000 diameters.

Fig. 103

Micrococcus tetragenus in peritoneal fluid. (After Zettnow.)

grayish-yellow disks, with a finely granular or mulberry-like surface, and a uniform but somewhat roughly dentated border. When the deep colonies push forward to the surface of the gelatin they form white, elevated, drop-like masses, having a diameter of 1 to 2 mm. In gelatin stick cultures the colonies may be either isolated or confluent, in the case forming a thick, white, slimy mass, filling out the fissures and hollow spaces all along the line of puncture; on the surface a broad, thick layer of 4 to 5 mm. in extent is apparent. The gelatin is not liquefied.

Growth on Agar and Blood Serum.—The colonies appear as small, transparent, round points, which have a grayish-yellow color and are slightly elevated above the surface of the medium.

Pathogenesis.—Subcutaneous injections of a culture of this micrococcus in minute quantity is usually fatal to white mice. The micrococci are found in comparatively small numbers in the blood of the vessels and heart, but are more numerous in the spleen, lungs, liver,

and kidneys. Intraperitoneal injections given to guinea-pigs and mice are followed by purulent peritonitis, beautifully formed cocci in groups of four being obtained in immense numbers from the exudate. Rabbits and dogs are not affected by large doses of a culture subcutaneously or intravenously administered.

The serum from immunized cases has not been used therapeutically in human infection.

THE STREPTOCOCCI.

Under this name must be included not only the streptococci which excite inflammation in man, but all spherical bacteria which divide, as a rule, in one plane only and hold cocci together in greater or lesser chains. This name comprises by no means so many varieties of bacteria as are grouped under the title bacilli. There are, nevertheless, a considerable number of distinct groups of streptococci which differ decidedly both in their cultural characteristics and their pathogenic properties. The streptococci average about 1μ in diameter. None of them form spores or are motile. They are rather easily killed by disinfectants. Those that are pathogenic develop wholly or almost so in or on the bodies of man and animals.

Streptococcus Pyogenes.—The group of streptococci which in its importance as related to human infections outweighs all other streptococci, is that which comprises the streptococci which excite erysipelas, many cases of cellulitis, abscess, septicæmia, pneumonia, etc., and passes under the name of *streptococcus pyogenes.*

This organism was first discovered by Koch in stained sections of tissue, attacked by septic processes, and by Ogston in the pus of acute abscesses (1882). It was obtained by Fehleisen (1883) in pure cultures from a case of erysipelas, its cultural and pathological characters studied and demonstrated by him to be capable of producing erysipelas in man. Rosenbach (1884) and Krause and Passet (1885) isolated the streptococcus from the pus of acute abscesses and gave it the name of streptococcus pyogenes. It has since been proved to be one of the chief etiological factors in the production of many suppurative inflammations. Formerly the streptococci of erysipelas, acute abscesses, septicæmia, puerperal fever, etc., were thought to belong to different species, because they were observed to possess apparent differences in their biological and pathological characteristics, according to the source from which they were obtained. Thus one species of streptococcus was believed to be capable of causing erysipelas only, another only acute abscesses, another sepsis, etc., but it is now known that the slight differences between the majority of these streptococci are but acquired variations of organisms derived from the same species which are not permanent.

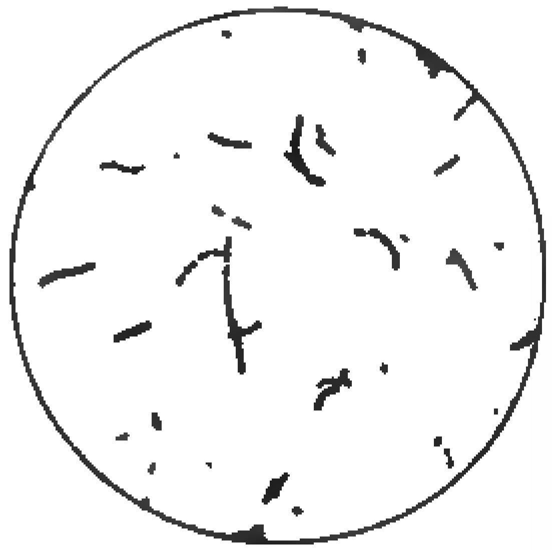
Morphology.—The cocci, when fully developed are spherical or oval. They have no flagella or spores. They vary from 0.4μ to 1μ in diameter. They vary in dimensions in different cultures and even in different

parts of a single colony. They multiply by binary division in one direction only, forming chains of eight, ten, twenty, and more elements, being, however, often associated distinctly in pairs. On solid media the cocci occur frequently as diplococci, but usually they grow in longer or shorter chains. Certain cocci frequently exceed their fellows greatly in size, especially in old cultures, when this may

FIG. 104

Streptococci in peritoneal fluid, partly enclosed in leukocytes. × 1000 diameters.

FIG. 105



Streptococci in throat exudate smeared on cover-glass. × 1000 diameters.

be considered to be the result of involution forms. Some varieties have distinct capsules when growing in the blood and in blood-serum media (Hiss).

FIG. 106

Streptococci from solidified serum culture appearing mostly in diplococci. × 1000 diameters.

FIG. 107

Streptococcus growing in long chains in bouillon culture. × 1000 diameters.

Staining.—They *stain* readily by aniline colors and the pyogenic varieties positively by Gram's method. Some varieties, mostly saprophytic, growing in short chains are negative to Gram's stain.

Biology.—Streptococci grow readily in various liquid and solid culture media. The most favorable temperature for their development is from 30° to 37° C., but they multiply rather freely at ordinary room-

temperature—18° to 20° C. They are facultative anaërobes, growing both in the presence and absence of oxygen.

Cultivation. GROWTH ON GELATIN.—Tubes of gelatin which have been inoculated with streptococci by puncture with the platinum needle show on the surface no growth beyond the point of entrance. In the depth of the gelatin on the second or third day a distinct, tiny band appears, with granular edges or made up of granules. These granules may be very fine or fairly coarse. They are nearly translucent, with a whitish, yellowish, or brownish tinge. With characteristic cultures the gelatin is *not liquefied,* though occasionally, with saprophytic varieties, a certain amount of liquefaction has been observed to take place.

GROWTH ON AGAR.—On agar plates the colonies are visible after twelve to thirty hours' growth at 37° C., and present a beautiful appearance when magnified sufficiently to see the individual cocci in the chain. The colonies are small, not averaging over 0.5 mm. in diameter (pin-head). From different sources they vary in size, thickness, mottling, color, and in the appearance of their borders. The streptococcus growing in short chains in bouillon shows but little tendency to form true loops, but rather projecting rows at the edges of the colonies, while those growing in long chains show beautiful loops, which are characteristic of this organism. The colonies are nearly circular in shape when thinly scattered over the plates, but irregular in form when crowded together.

GROWTH IN BOUILLON.—Most streptococci grow well in slightly alkaline bouillon at 37° C., reaching their full development within thirty-six to forty-eight hours. Those which grow in long chains usually give an abundant flocculent deposit and leave the liquid clear. The deposit may be in grains, in tiny flocculi, in larger flakes, or in tough, almost membranous masses, the differences depending on the strength of union between the pairs of cocci in the chains. Some of the streptococci growing in long chains, however, cause the broth to become cloudy. This cloudiness may be only temporary or it may be lasting. Those growing in short chains, as a rule, cloud the broth, this cloudiness remaining for days or weeks. A granular deposit appears at the bottom of the tube. An addition of 0.5 to 1 per cent. glucose aids the development of streptococci, but the acid produced tends later to hasten their death and make them lose virulence. A trace of calcium aids the growth. This is best added as a piece of marble, which has the additional advantage of neutralizing some of the acids produced.

GROWTH IN ASCITIC OR SERUM BOUILLON.—The development in this, which is the best medium for the growth of the streptococcus, is more abundant than in plain bouillon. The liquid is clouded, and a precipitate only occurs after some days, the fluid gradually clearing. The addition of blood serum frequently causes streptococci, growing in short chains in nutrient bouillon, to produce long chains. The reverse is also true, and in the blood all forms are usually found, partly, at least, as diplococci or in short chains.

EFFECT ON INULIN.—This is not fermented by most varieties.

Growth on Solidified Blood Serum.—This is also an excellent medium for the streptococcus. Tiny, grayish colonies appear twelve to eighteen hours after inoculation.

Growth in Milk.—All streptococci grow well in milk. As a rule, when growth is luxuriant a marked production of lactic acid with coagulation of the casein occurs.

Development in Blood Agar.—Most streptococci produce abundant hæmolytic substances. This is especially true of those from human septic infections. As the pneumococci and other types of streptococci produce them in a much less degree, blood-agar plates are a very useful means for a probable identification. According to Rosenow if 1 c.c. of fresh or defibrinated blood is added to 6 c.c. of melted agar at 40° to 45° C., well shaken, inoculated with the organisms and poured in a Petri dish there will appear in twelve to twenty-four hours, if characteristic streptococci are present, tiny colonies surrounded by clear zones of about $\frac{1}{4}$ to $\frac{1}{3}$ inch in diameter. Pneumococci and some varieties of streptococci on the other hand produce only narrow zones, but instead a green pigment.

Duration of Life Outside of the Body.—This is not, as a rule, very great. When dried in blood or pus, however, they may live for several months at room-temperature, and longer in an ice-chest, and in gelatin and agar cultures they live for from one week to three months. In order to keep streptococci alive and virulent, it is best to transplant them frequently and to keep them in serum or ascitic fluid bouillon in small, sealed glass tubes in the ice-chest.

Resistance to Heat and Chemicals.—The thermal death point of the streptococcus is between 52° and 54° C., the time of exposure being ten or twenty minutes.

Mercuric chloride, 1:2500; sulphate of copper, 1:200; trichloride of iodine, 1:750; peroxide of hydrogen, 1:50; carbolic acid, 1:300; cresol, 1:250; lysol, 1:300; creolin, 1:130, all kill streptococci within a few minutes.

Pathogenesis.—The majority of test animals are not very susceptible to infection by the streptococcus, and hence it is difficult to obtain any definite pathological alterations in their tissues through the inoculation into them of cultures of this organism by any of the methods ordinarily practised. White mice and rabbits, under similar conditions, are the most susceptible, and these animals are, therefore, usually employed for experimentation. Streptococci, however, differ greatly in the effects which they produce in inoculated animals, according to their animal virulence, which is very different from human virulence. The most virulent when injected in the minutest quantity into the circulation or into the subcutaneous tissues of a mouse or rabbit, produce death by septicæmia. Those of somewhat less virulence produce the same result when injected in considerable quantities. Those still less pathogenic produce septicæmia, which may be mild or severe, when injected into the circulation; but when injected subcutaneously, they produce abscess or erysipelas. The remaining streptococci, unless introduced in quan-

tities of 20 c.c. or over, produce only a slight redness, or no reaction at all, when injected subcutaneously, and little or no effect when injected directly into the circulation. Many of the streptococci obtained from cases of cellulitis, abscess, empyema, and septicæmia belong to this group.

A number of varieties of streptococci have thus been discovered, differing in virulence and in their growth on artificial media; but all attempts to separate them into various classes, even with the use of specific serum, have largely failed, because the differences observed, though often marked, are not constant, many varieties having been found to lose their distinctive characteristics, and even to apparently change from one class to another. A further objection to any of the existing classification of streptococci, which are based on the manner of growth on artificial culture media, is that it has been impossible to make any which would at the same time give even an approximate idea of their virulence. Experiments have proved that streptococci originally virulent may become non-virulent after long cultivation on artificial media, and, again, that they may return to their original properties after being passed through the bodies of susceptible animals. The peculiar type of virulence which they may acquire tends to perpetuate itself, at least for a considerable time.

One important fact that experience teaches us is, that those streptococci which are the most dangerous are those which have come immediately from septic conditions, and the more virulent the case the more virulent the streptococci are apt to be for animals of the same species. There seems also to be a strong tendency for a streptococcus to produce the same inflammation, when inoculated, as the one from which it was obtained; for example, streptococci from erysipelas tend to produce erysipelas, from septicæmia to produce septicæmia, etc. Streptococci, however, obtained from different sources (abscesses, puerperal fever, sepsis, erysipelas, etc.) are in many instances capable, under favorable conditions, of producing erysipelas when inoculated into the ear of a rabbit, as has been proved by experiment, provided they possess sufficient virulence.

Occurrence in Man.—Streptococci have been found in man as the primary cause of infection in the following diseases: Erysipelas, circumscribed and extensive acute abscesses, impetigo, cellulitis (circumscribed as well as diffused), sepsis, puerperal infection, lymphatic abscesses, angina, bronchopneumonia, periostitis, osteomyelitis, synovitis, otitis media, mastoiditis, meningitis, pleurisy, empyema, and endocarditis. Associated with other bacteria in diseases of which they were the specific cause, they have also been found as the secondary or mixed infection in many diseases, such as in pulmonary tuberculosis, bronchopneumonia, septic diphtheria, and diphtheritic scarlatina. In diphtheritic false membranes this micrococcus is very commonly present, and is frequently the source of deeper infection, such as abscesses and septicæmia; and in certain cases attended with a diphtheritic exudation, in which the Loeffler bacillus has not been found by competent bacteriologists, it seems probable that the streptococcus pyogenes, alone or with other

pyogenic cocci, is responsible for the local inflammation and its results. These forms of so-called diphtheria, as first pointed out by Prudden, are most commonly associated with scarlatina and measles, erysipelas, and phlegmonous inflammation, or occur in individuals exposed to these or other infectious diseases. So uniformly are streptococci present in the pseudomembranous inflammation of patients sick with scarlet fever, that many investigators have suspected a special variety of them to be the cause of this disease. The same is true for smallpox. Many varieties are regularly found, however, in the throat secretion of healthy individuals (in 100 examinations by us we found them in 83, and probably could have found them in some of the others by longer search). Their presence in scarlet fever and smallpox is most probably due to their increase in the disordered mucous membrane and entrance into the circulation when the protective properties of the blood have been lowered.

The causal relation of the streptococcus to the above-mentioned diseases has been amply proved by inoculation experiments both in man and animals. Fehleisen has inoculated cultures, obtained in the first instance from the skin of patients with erysipelas, into patients in the hospital suffering from inoperable malignant growths—lupus, carcinoma, and sarcoma—and has obtained positive results, a typical erysipelatous inflammation having developed around the point of inoculation after a period of incubation of from fifteen to sixty hours. This was attended with chilly sensations and an elevation of temperature. Persons who had recently recovered from an attack of erysipelas proved to be immune. These experiments were undertaken on the ground that malignant tumors had previously been found to improve or entirely disappear in persons who had recovered from accidental erysipelas. During the last few years this fact has been therapeutically applied to the treatment of malignant tumors by the artificial production of erysipelas by the inoculation of pure cultures of streptococcus or of their toxic products.

Results from Injections in Tumors.—In some cases of sarcoma this method has met with considerable success; in carcinoma, however, the results have been very slight. In this country the experimental work upon this subject and the actual treatment of cases have been largely carried out by or under the direction of Coley. He has kindly sent me the following notes on his results:

"The improvement and inhibitory action which the toxins have upon carcinoma have proved to be, in nearly all cases, but temporary.

"On the other hand, in sarcoma, which is the only form of malignant tumor in which I have advocated the treatment, sufficient time has elapsed to enable us to draw the following conclusions:

"The toxins injected subcutaneously into the tissues, either into the tumor substance or into parts remote from the tumor, exercise a distinctly inhibitory action upon the growth of nearly all varieties of sarcoma. This action is the least marked in melanotic sarcoma, and thus far no cases of this form of tumor have disappeared under the treatment. The influence of the toxins upon round-celled sarcoma is much more powerful than it is upon melanotic, although distinctly less than upon the spindle-

celled variety. A number of cases of round-celled sarcoma in which the diagnosis was unquestioned disappeared, and the patients have remained well beyond three years. Nearly half of the cases treated showed no appreciable decrease in size; the majority of the others which did show marked improvement at first, after decreasing in size for a few weeks, again began to increase and were no longer influenced by the treatment.

"In half of the cases of spindle-celled sarcoma treated by the toxins the disease had disappeared entirely, and the majority of the successful cases have remained well sufficiently long to justify their being regarded as cured. It should be distinctly stated that all of the tumors under consideration were inoperable, as I have never advised treatment except in such cases.

"I have now a number of cases of spindle-celled sarcoma which have remained well beyond three years; one case of mixed (round and spindle) celled, after remaining well three years, had a return in the abdomen, and died about eight months later. The result here certainly establishes the correctness of the early diagnosis."

Production of Toxic Substances.—There is no doubt that the streptococcus causes fever, general symptoms of intoxication, and death by means of toxic substances which it forms in its growth; but we know but little about these substances or how they are produced. The poisons while partly extracellular are mostly contained in the cell substance. Heat destroys a portion of them. They appear to attack especially the red blood cells, and this hæmolytic action seems to be to some degree in proportion to the virulence of the organism.

Susceptibility to Streptococcus Infection.—As with the ever-present staphylococci, whose virulence, as we have seen, is usually slight, the streptococci are more likely to invade the tissues, forming abscesses or erysipelatous and phlegmonous inflammation in man when the standard of health is reduced from any cause, and especially when by absorption or retention various toxic organic products are present in the body in excess. It is thus that the liability to these local infections, as complications of operations or sequelæ of various specific infectious diseases, in the victims of chronic alcoholism, and constitutional affections, etc., are to be explained. It seems established that the absorption of toxic products formed in the alimentary canal as a result of the ingestion of improper food, or in consequence of abnormal fermentative changes in the contents of the intestine, or from constipation, predispose to infection.

Immunity.—Knorr succeeded in producing a moderate immunity in rabbits against an intensely virulent streptococcus by injections of very slightly virulent cultures. Pasquale was able to immunize these animals partially against septicæmia. Marmorek has immunized sheep, asses, and horses against very large doses of a streptococcus, which though but slightly virulent for them was intensely so for rabbits.

In none of the streptococcus inflammations do we notice much apparent tendency to the production of immunizing and curative substances in the blood by a single infection.

Severe general infections usually progress to a fatal termination

after a few days, weeks, or months. It is true, however, that cases of erysipelas, cellulitis, and abscess, after periods varying from a few days to months, tend to recover, and to a certain extent, therefore, we may assume that protective agents have been produced. In these cases, however, we know from experience that faulty treatment, by lessening the local or general resistance, would, as a rule, cause the subsiding infection to again progress perhaps even to a more serious extent than the original attack. Koch and Petruschky tried a most interesting experiment. They inoculated cutaneously a man suffering from a malignant tumor with a streptococcus obtained from erysipelas. He developed a moderately severe attack, which lasted about ten days. On its subsidence they re-inoculated him; a new attack developed, which ran the same course and over the same area. This was repeated ten times with the same results.

This experiment proved that in this case, at least, little if any lasting curative or immunizing substances were produced by repeated attacks of erysipelas, and that the recovery from each attack was due to local and transitory protective developments.

The severe forms of infection, such as septicæmia following injuries, operations, and puerperal infections, show little tendency to be arrested after being well established. Having, then, in remembrance, the above facts, let us consider the results already obtained in the experimental immunization and treatment of animals and men suffering from or in danger of infection with streptococci. One method is now chiefly used for the immunization and attempt to produce curative substances in animals, namely the injection in gradually increasing doses of the living, virulent streptococcus itself. Marmorek was the first to attempt the production of a curative serum on a large scale.

Influence of Serum from Immunized Animals upon Streptococcus Infections in Other Animals.—In the table are given the results following the injection of small amounts of a serum which represents in immunizing value what about one-third of the horses are able to produce. In the following experiments the serum and culture were injected subcutaneously in rabbits at the same time, but in opposite sides of the body:

TABLE *Showing Strength of Average Grade of Antistreptococcic Serum given by Selected Horses after six months of Injection of suitabe amounts of Living Streptococci.*

	Weight of rabbit.	Amounts inoculated.		Result.	Autopsy.
Serum and culture:	Grms.	Serum.	Cult.		
1. Inoculated together	1430	0.25 c.c.	0.01 c.c.	Lived	
2. " "	1350	0.125 "	0.01 "	"	
3. On opposite sides	1770	0.1 "	0.01 "	"	
4. " "	1630	0.1 "	0.01 "	"	
Controls:					
1. Rabbits injected with culture only	1750	....	0.001 "	Died in 4 days.	Streptococcic infection.
2. " " " "	1870	....	0.001 "	Died in 24 hrs.	Streptococcic infection.

The above results have been repeatedly obtained, and are absolutely conclusive that the serum of properly selected animals, which have been repeatedly injected with living streptococci in suitable doses, possesses bactericidal properties upon the same streptococcus when it comes in contact with it within the bodies of animals.

Definite protection from the serum has been obtained by many reliable observers since Marmorek's first reports.

Is Protection Afforded by the Same Serum against all Varieties of Streptococci?—We have tested the protective value of one serum against streptococci derived from five different sources. First, the streptococcus given us by Marmorek, which was obtained from a case of angina. Its virulence is now such, after having passed through hundreds of rabbits, that 0.000001 c.c. is the average fatal dose. Second, a streptococcus obtained from a case of erysipelas in England. Its virulence is 0.00001 c.c. on the average. Third, a streptococcus obtained from a case of cellulitis, its virulence being about 6 c.c. Fourth, a streptococcus sent me by Theobald Smith. Its virulence is such that 0.1 c.c. is the average fatal dose. Fifth, another culture sent me by Smith, which grew in short chains and was obtained from milk; its virulence was similar to No. 4.

Against the first three streptococci derived from three different varieties of infection existing in three different countries the serum produced by the streptococcus from England had nearly the same value. Against the latter two streptococci, as well as against a streptococcus from a case of endocarditis, which resembled in some respects the pneumococci and a pneumococcus, the serum had no effect.

The results of numerous investigators indicate that the majority of streptococci met with in septic infections will be influenced by the same serum. Many more streptococci, however, must be obtained from human infections and tested before we can be certain of this. Those obtained from cases of pneumonia and endocarditis which have some resemblance to pneumococci and which are not very virulent in animals, are especially in need of investigation.

Preparation of the Serum.—Antistreptococcus serum is obtained from the horse after treatment by repeated injections of living streptococcus cultures of streptococci derived from human sources. As a rule, a number of varieties are given at the same time so that the serum will be active against any variety causing the infection. If the serum is to be used in scarlet fever, the streptococci used should be from cases of scarlet fever. The procuring of a serum of the highest potency requires a considerable number of animals, for some produce with the same treatment a more protective serum than others. The serum must be sterile from streptococcus as well as from other contaminations.

Stability of the Serum.—Unfortunately, after several weeks or months, the serum, as a rule, loses most of its protective value. It should be kept in a cold and dark place.

Standardization of the Value of the Serum.—The value of the serum is measured by the amount required to protect against a multiple of a fatal

dose of a very virulent streptococcus. The dose is usually a thousand times the average fatal amount of a very virulent streptococcus.

This method gives, as a rule, to those unfamiliar with bacteriology, an exaggerated idea of the potency of the serum.

A thousand times the amount of a very virulent streptococcus culture required to kill an animal by producing septicæmia is more easily controlled than four times a fatal dose of a slightly virulent streptococcus. The serum acts upon a certain quantity of organisms, while it is only their enormous multiplication in the animal which kills.

It is entirely different in case of an antitoxin which does not prevent primarily the growth of the germ, but neutralizes a chemical substance —its toxin.

Therapeutic Results.—To estimate the exact present and future value of antistreptococcus serum is a matter of the utmost difficulty. Many of the cases reported are of little or no help, because no cultures having been made, we are in doubt as to the nature of the bacterial infection.

Marmorek's results are by far the best reported, but without casting any doubt upon the justification of his conclusions, from the data at his command, I believe they undoubtedly give too favorable a view of the value of the serum.

In the few cases of puerperal fever, erysipelas, wound infection, scarlet fever, and bronchopneumonia that we have seen, the apparent results under the treatment have not been uniform. Only occasionally have we seen results which appeared to be distinctly due to the serum.

In a number of cases of septicæmia where for days chills had occurred daily they ceased absolutely or lessened under daily doses of 20 to 50 c.c. The temperature, though ceasing to rise to such heights, did not average more than one or two degrees lower than before the injections. In some cases the serum treatment was kept up for four weeks. Some cases convalesced; others after a week or more grew worse and died. In some cases the temperature fell immediately upon giving the first injection of serum, and after subsequent injections remained normal, and the cases seemed greatly benefited. As a rule, in these cases no streptococci or any other organisms were obtained from the blood. In bronchopneumonia, laryngeal diphtheria, scarlet fever, smallpox, and phthisis, we have seen absolutely no effect. In the exanthemata our injections were much smaller than those used in Vienna, in which city very striking results are reported from 100 c.c. doses.

The results obtained here in New York by both physicians and surgeons have not, on the whole, been very encouraging.

In some of the cases where apparently favorable results were obtained other bacteria than streptococci were found to be the cause of the disease. We believe that the following conclusions will be found fairly accurate:

A single antistreptococcic serum protects healthy rabbits from infection from most of the streptococci obtained from human sepsis, but

not from all. Failure to do good in human infection cannot, as a rule, be attributed to the variety of streptococcus. The serum will in animals limit an infection already started if it has not progressed too far. The apparent therapeutic results in cases of human streptococcus infection are variable. In some cases the disease has undoubtedly advanced in spite of large injections, and here it has not seemed to have had any effect. In other cases good observers rightly or wrongly believe they have noticed great improvement from it. Except rashes, few have noticed deleterious results, although very large doses have been followed in several instances, for a short time, by albuminous urine and even temporary suppression.

In suitable cases we are warranted, we believe, in trying it, but we should not expect very striking results.

For our own satisfaction, and to increase our knowledge, we should always have satisfactory cultures made when possible, and the streptococci, if obtained, tested with the serum used in the treatment. In the cases where we want most to use the serum, such as puerperal fever, septicæmia, ulcerative endocarditis, etc., we find that it is very difficult to make a bacteriological diagnosis from the symptoms, and in over one-half of the cases even the bacteriological examination carried out in the most thorough way will fail to detect the special variety of bacteria causing the infection. This is often a great hindrance to the proper use of curative antistreptococcic serum, for it, of course, has no specific effect upon the course of any infection except that due to the streptococcus and the full effect only on its own type.

Care should be taken to get only recently tested serum, for after six weeks the best serum is almost inert; much on the market is worthless, and as it is weak, and the testing for strength is still very crude, full doses (10 to 20 c.c.) of serum should be given if the case is at all serious, for the dose is limited only by the amount of horse serum which we feel it safe to give, not because we have sufficient protective substance.

Bacteriological Diagnosis.

Streptococci, using the name in a broad sense, can often be demonstrated microscopically by simply making a smear preparation of the suspected material and staining with methylene-blue solution or diluted Ziehl's fluid. In order to demonstrate them microscopically in the tissues, the sections are best stained by Kühne's methylene-blue method. In all cases, even when the microscopic examination fails, the cocci may be found by the culture method on plate agar or slanted agar at 37° C. To obtain them from a case of erysipelas it is best to excise a small piece of skin from the margin of the erysipelatous area in which the cocci are most numerous; this is crushed up and part of it transferred to ascitic or serum bouillon, and part is streaked across freshly solidified agar in a Petri dish on which a drop of sterile rabbit's blood had previously been placed. Both are kept in the incubator at 37° C.

In septicæmia the culture method is always required to demonstrate the presence of streptococci, as the microscopic examination of specimens of blood is not sufficient. For this purpose from 10 to 15 c.c. of the blood should be drawn from the vein of the arm aseptically by means of a hypodermic needle, and to each of three tubes containing 10 c.c. of melted nutrient agar kept at about 43° C. 1 c.c. of blood is added. After thoroughly mixing the contents are poured into Petri dishes. The remainder is added to flasks or tubes of nutrient broth, in order to produce an adequate development of the cocci, which are found in small numbers in the bloodvessels. Petruschky is of the opinion that the cocci can best be shown in blood by animal inoculation. Having withdrawn from the patient 10 c.c. of blood by means of a hypodermic syringe, under aseptic precautions, he injects a portion of this into the abdominal cavity of a mouse, while the other portion is planted in bouillon. Mice thus inoculated die from septicæmia when virulent streptococci are present only in very small numbers in the blood. If a successful inoculation takes place we can, through the absence or presence of the development of capsules, often differentiate between the pneumococcus and the streptococcus, which cultures may fail to do. The development of a wide, clear zone about the colonies, without a development of green pigment, indicates that the streptococci belong to the pyogenes type. The absence of a definite zone and the development of a green color indicates that they are pneumococci or streptococci which in these two respects resemble pneumococci. The growth in the Hiss inulin serum medium will generally differentiate between the two, as the pneumococci usually coagulate the serum, while the great majority of streptococci do not. The morphological and cultural characteristics of the streptococcus give us, unfortunately, no absolute knowledge as to the influence which the protecting serum will have. The actual test is here our only method. The detection of the streptococcus in the blood is in itself an unfavorable prognostic sign.

The blood cultures in many cases of septicæmia give no positive results, for many of these cases develop their symptoms and even die from the absorption of toxins from the local infection, such as an amputation wound or an infected uterus or peritoneum, and the bacteria never invade the blood. When we get negative results we are, as a rule, utterly unable to test the case with curative serums with any accuracy, for the sepsis may be due to either the streptococcus, colon bacillus, staphylococcus, or a number of other pathogenic varieties of bacteria.

CHAPTER XXV.

THE DIPLOCOCCUS OF PNEUMONIA (PNEUMOCOCCUS, STREPTOCOCCUS LANCEOLATUS, MICROCOCCUS LANCEOLATUS). THE PNEUMOBACILLUS (FRIEDLÄNDER BACILLUS).

The Diplococcus of Pneumonia.

THE diplococcus of pneumonia was observed in 1880 almost simultaneously by Sternberg and Pasteur in the blood of rabbits inoculated with human saliva. In the next few years Talamon, Friedländer, A. Fraenkel, Weichselbaum, and others subjected this micro-organism to an extended series of investigations and proved it to be the chief etiological factor in the production of lobar or croupous pneumonia in man.

The outcome of the various investigations proved that the acute lung inflammations, especially when not of the frank lobar pneumonia type, are not excited by a single variety of micro-organism, and that the bacteria involved in the production of pneumonias are also met with in inflammations of other tissues.

In any individual pneumonic inflammation it is also found that more than one variety of bacteria may be active, either from the start or as a later addition to the original primary infection.

Among all the micro-organisms active in exciting pneumonia, the diplococcus of pneumonia is by far the most common, being almost always present in primary lobar pneumonia and as frequently as any other germ in acute bronchopneumonia and metastatic forms. Besides the different varieties of pneumococci the following bacteria are capable of exciting pneumonia: streptococcus pyogenes, staphylococcus pyogenes, bacillus pneumoniæ, bacillus influenzæ, bacillus pestis, bacillus diphtheriæ, bacillus typhi, bacillus coli, and the bacillus tuberculosis. Since the varieties of bacteria exciting acute pneumonia, with the exception of the pneumococcus, are met with more frequently in other inflammations and have been described elsewhere, they will only be noticed in this chapter so far as their relation to pneumonia demand.

Morphology.—Typically, the pneumococcus occurs as spherical or oval cocci, usually united in pairs, but sometimes in longer or shorter chains consisting of from three to six or more elements and resembling the streptococcus. The cells, as they commonly occur in pairs, are somewhat oval in shape, being usually pointed at one end—hence the name *lanceolatus* or lancet-shaped. When thus united the junction, as a rule, is between the broad ends of the oval, with the pointed ends turned outward; but variation in form and arrangement of the cells is characteristic of this

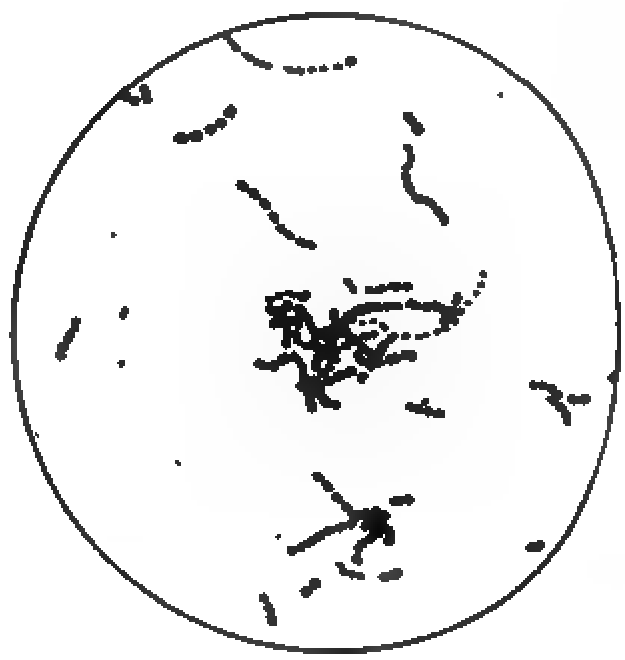
organism, there being great differences according to the source from which they are obtained. As observed in the sputum and blood it is usually in pairs of lancet-shaped elements, which are surrounded by a capsule. (See Fig. 108.) When grown in fluid culture media longer or shorter chains are frequently formed, which can scarcely be distinguished from chains of certain streptococci, except that, as a rule, the length of the chain is less and the pairs of diplococci are farther apart. In cultures the individual cells are almost spherical in shape, and except in certain varieties are rarely surrounded by a capsule. (See Fig. 109.) The pneumococcus is by some classed as a streptococcus.

The capsule is best seen in stained preparations from the blood and exudates of fibrinous pneumonia or from the blood of an inoculated animal, especially the mouse, in which it is commonly, though not

FIG. 108

Diplococcus of pneumonia from blood, with surrounding capsule stained by method of Hiss.

FIG. 109



Pneumococcus from bouillon culture, resembling streptococcus.

always, present. It is seldom seen in preparations from cultures unless special media are employed. Flagella are not present.

Staining.—It *stains* readily with ordinary aniline colors; it is not decolorized after staining by Gram's method. The capsule may be demonstrated in blood or sputum either by Gram's or Welch's (glacial acetic acid) method, or the copper sulphate method of Hiss.

Biology.—It grows equally well with or without oxygen, being thus both aërobic and facultative anaërobic; its parasitic nature is exhibited by the short range of temperature at which it usually grows—viz., from 25° to 42° C.—best at 37° C. In the cultivation of this organism neutral or slightly alkaline media should be employed. The organism grows feebly on the serum-free culture media ordinarily employed for the cultivation of bacteria viz., on nutrient agar and gelatin, in bouillon. The best medium for its growth is a mixture of one-third

human or animal blood serum or ascitic or pleuritic fluid and two-thirds bouillon or nutrient agar growth in milk.

GROWTH IN MILK.—It grows readily in milk, causing coagulation with the production of acid, though this is not constant with some forms intermediate between the streptococcus and pneumococcus.

GROWTH ON AGAR.—Cultivated on plain nutrient agar, after twenty-four to forty-eight hours at 37° C., the deep colonies are hardly visible to the eye. Under the microscope they appear light yellow or brown in color and finely granular. The surface colonies are large, equalling in size those of streptococci, but are usually more transparent. If blood serum or ascitic fluid be added to the agar the individual colonies are larger and closer together, and the growth is more distinct in consequence and of a grayish color. The surface colonies are almost circular in shape under a magnification of 60 diameters, finely granular in structure, and may have a somewhat darker, more compact centre, surrounded by a paler marginal zone. With high magnification cocci in twos and short rows often distinctly separated are seen at the edges. In stick cultures minute transparent drops appear along the line of puncture.

GROWTH ON GELATIN.—The growth on gelatin is slow, if there is any development at all, owing to the low temperature—viz., 24° to 27° C.—above which even the most heat-resistant gelatin will melt. The gelatin is not liquefied.

GROWTH ON BLOOD SERUM.—The growth on Loeffler's blood-serum mixture is very similar to that on agar, but somewhat more vigorous and characteristic, appearing on the surface as a delicate layer of dew-like drops.

GROWTH IN BOUILLON.—In bouillon, at the end of twelve to twenty-four hours in the incubator, a slight cloudiness of the liquid will be found to have been produced, due to the development of the micrococci. On microscopic examination these can be seen to be arranged in pairs or longer or shorter chains. In two or three days the medium again becomes transparent owing to the subsidence of the cocci. After one or two transplantations the pneumococci frequently fail to grow.

SPECIAL MEDIA.—Fraenkel was the first to draw attention to the fact that this organism soon loses its reproductive power when grown on ordinary culture media, and more particularly solid media. In fluid media the vitality is not quite so quickly lost; but even here it is found advisable to transplant fresh cultures every day. When cultures are grown on serum-free media the vitality of some cultures may indeed be indefinitely prolonged; but after transplantation through several generations it is found that the cultures begin to lose in virulence, and that they finally become non-virulent. In order to restore this virulence, or to keep it from becoming attenuated, it is necessary to interrupt the transplantation and pass the organism through the bodies of susceptible animals.

With the view of overcoming these obstacles in the way of cultivating this micrococcus, several special media have been proposed by various experimenters in the place of the ordinary culture media. The

best fluid medium for the growth of the pneumococcus is Marmorek's mixture, consisting of bouillon 2 parts and ascitic or pleuritic fluid 1 part. In this fluid pneumococci grow well, and cultures when preserved in a cool place and prevented from drying retain their vitality and also their virulence for a number of weeks. Lambert has found cultures in this medium alive and fully virulent after eight months.

Loeffler's blood-serum mixture is a good, solid tube medium for making cultures, and is very convenient and useful at autopsies. One-and-one-half per cent. fluid nutrient agar mixed with one-third its quantity of warm ascitic fluid makes an excellent plate medium.

Hiss Serum Media with and without Inulin.—These are very useful. The inulin is fermented by typical pneumococci with coagulation of the serum. While most streptococci fail to ferment the inulin. This medium is, therefore, of considerable diagnostic value,

Nutrient agar, with fresh rabbit blood smeared over it makes an excellent medium for growth, but prevents the development of agglutinable substance. On this medium, in a moist atmosphere at 36° C., the cultures retain their viability and virulence for rabbits for months,[1]

Calcium Broth with or without Dextrose.—This medium has proven of great value for the propagation of cultures where agglutination tests are to be carried out. The addition of a small piece of marble to each tube of broth is the most satisfactory way of preparing it. Marble broth for this purpose was suggested independently by Bolduan and Hiss.

Resistance to Light, Drying, and Germicidal Agents.—On artificial culture media the pneumococci tend to die rapidly. This is partially due to the acid produced by their growth. In sputum they live much longer.

Pneumonic sputum attached in masses to clothes, when dried in the air and exposed to diffuse daylight, retains its virulence, as shown by injection in rabbits, for a period of nineteen to fifty-five days. Exposed to direct sunlight the same material retains its virulence after but a few hours' exposure. This retention of virulence for so long a time under these circumstances is accounted for by the protective influence afforded by the dried mucoid material in which the micrococci were embedded. Guarnieri observed that the blood of inoculated animals, when rapidly dried in a desiccator, retained its virulence for months; and Foá found that fresh rabbit blood, after inoculation and cultivation in the incubator for twenty-four hours, when removed at once to a cool, dark place, retained its virulence for sixty days. There are many conditions, therefore, in which the virulence of the micrococcus is retained for a considerable length of time. To germicidal agents pneumococci are very sensitive. The fine spray expelled in coughing and loud speaking soon dries so completely that no pneumococci usually survive after two hours. The same is true for dust that remains suspended in the air.

[1] The green color produced by all pneumococci in blood-agar media, and showing especially well in poured blood-agar plates is not diagnostic of this organism, as some strains of streptococci produce just as intense a green.

Pathogenesis in Animals.—Most strains of the micrococcus lanceolatus are moderately pathogenic for numerous animals; mice and rabbits are the most susceptible, indeed some strains are intensely virulent for these animals, while guinea-pigs and rats are much less susceptible. Pigeons and chickens are refractory. In mice and rabbits the subcutaneous injection of small or moderate quantities of pneumonic sputum in the early stages of the disease, or of a twenty-four hour ascitic broth culture from such sputum, or of a pure, virulent ascitic broth culture of the micrococcus, usually results in the death of these animals in from twenty-four to forty-eight hours. The course of the disease produced and the post-mortem appearances indicate that it is a form of septicæmia—what is known as sputum septicæmia. After injection there is loss of appetite and great debility, and the animal usually dies some time during the second day after inoculation. The post-mortem examination shows a local reaction, which may be of a serous, fibrinous, hemorrhagic, necrotic, or purulent character; or there may be combinations of all of these conditions. The blood of inoculated animals immediately after death often contains the micrococci in very large numbers. For microscopic examination they may be obtained from the blood of the veins, arteries, or cavities of the heart, and usually from the pleural and peritoneal exudations when these are present.

True localized pneumonia does not usually result from subcutaneous injections into susceptible animals, but injections made through the thoracic walls into the substance of the lung may induce a typical fibrous pneumonia. This was first demonstrated by Talamon, who injected the fibrinous exudate of croupous pneumonia, obtained after death or drawn during life from the hepatized portions of the lung, into the lungs of rabbits. Wadsworth showed that by injecting virulent pneumococci into the lungs of rabbits which had been immunized, a typical lobar pneumonia was excited, the general bactericidal property of the blood being sufficient to prevent the general invasion of the bacteria.

Attenuation of Virulence.—This may be produced in various ways. The loss of virulence which occurs when the micrococcus is transplanted through several generations in culture fluid containing no blood has already been referred to. An apparent attenuation of virulence takes place also spontaneously in the course of pneumonia. It has been shown by daily puncture of the lung in different stages of the pneumonic process that the virulence of the organism diminished as the disease progresses, and that the nearer the crisis was approached the more attenuated it became. This attenuation is probably only apparent. So many more micro-organisms are living in each cubic centimetre of fluid during the early stages of a pneumonia that much smaller quantities kill. If a little sputum be taken at different periods in the disease and planted in ascitic bouillon the resultant cultures will not vary greatly in virulence. The average virulence of cultures made from pneumonic sputum is greater than in those from normal sputum.

Restoration and Increase of Virulence.—The simplest and perhaps the most reliable method of restoring lost virulence for any susceptible

animal is by passage through the bodies of highly susceptible animals of the same species. Growth in fresh blood also increases it for the homologous animal.

Toxin Production.—We have little exact knowledge upon the nature of the substances produced by or through the growth of the pneumococci in animal tissues or artificial media.

Occurrence in Man during Health.—It is probable that in crowded communities the pneumococcus is present on the mucous membranes of most persons. We have found it generally present not only in the throats of persons living in New York City, but also in those of persons living on farms and in the Adirondack Mountains. It is commonly present only on the mucous membranes of the bronchi, trachea, pharynx, and nostrils. The healthy lung seems to be generally free from it. Judging from animal tests it is very possible that the virulence for man of the organisms present in health is much less than the virulence of those in a pneumonic lung.

Presence of Pneumococci in Lobar and Bronchopneumonia.—Fully 95 per cent. of characteristic cases of lobar pneumonia are due primarily to characteristic or atypical pneumococci. Usually no other bacteria are obtained from the lungs. Atypical cases are frequently due to pneumococci, but they may be due to streptococci, influenza bacilli, etc.. The more recent the infection the greater is the number of bacteria found in the diseased lung area. As the disease progresses these decrease in number until finally at the crisis they disappear from the tissues, though at this time and long after convalescence they may be present in the sputum. In atypical forms of pneumonia they may remain longer in the tissues, and in walking pneumonia they may be absent in the original centres of infection or present only as attenuated varieties, while the surrounding, newly formed foci may contain fully virulent cocci. It has been shown by Netter that more than one-half of the cases of bronchopneumonia, whether primary, or secondary to some other disease, as measles and diphtheria, both in children and adults, are due to the diplococcus of pneumonia. Others, such as Pearce, have found other micro-organisms, especially the streptococci, in the majority of cases. These findings will be considered at the end of the chapter.

The pneumococci are found partly in the alveoli and bronchioles of the inflamed lung and partly in the lymph channels and blood capillaries. Most of the organisms are found free, but a few are found in the leukocytes. Through the lymph channels they find their way to the pleura and to adjacent lymph glands. From the capillaries they find their way to the general blood current, and thus to distant parts of the body. In about 20 per cent. of cases the pneumococci are so abundant that they can be found in cultures made from 5 to 10 c.c. of blood. In a number of instances the fœtus has been found infected.

Occurrence in Inflammations Complicating Pneumonia.—In every case of lobar pneumonia and in most cases of bronchopneumonia pleurisy is developed, which is excited by the same micro-organism that was

predominant in the pneumonia. With pneumococci the exudate is usually moderate and of a fibrinous character, but may be more abundant and of a serofibrinous or purulent character. When the pleurisy is marked it is more apt to continue after the cessation of the pneumonia. Pleurisy due to pneumococci is more apt to go on to spontaneous recovery than that due to streptococci or staphylococci.

The most frequent pneumococcic infections next to pleurisy, following a pneumonia, are those of the pericardium, endocardium, and meninges, and these not infrequently arise together. Pneumococcic inflammations of the heart valves are apt to be followed by extensive necrosis and growth of vegetations. In these cases pneumococci can sometimes be found in the blood for many weeks. Pericarditis due to pneumococci is a frequent complication, but is usually very slightly developed. Meningitis due to pneumococci may be either fibrinous or purulent or both. Arthritis and periarthritis are rarer complications of a pneumococcic pneumonia. Besides moderate parenchymatous inflammation of the kidney, which occurs in most cases of pneumonia, well-marked inflammation may occur in which pneumococci exist in the kidney tissues in large numbers. Osteomyelitis and otitis media are not very infrequent.

How is the pneumococcus conveyed from its original seat in the lungs to distant internal organs? Chiefly by means of the bloodvessels and lymphatics, in both of which it has been found in great numbers. Proof enough of its conveyance through the lymphatics is afforded by the frequent occurrence of inflammations of the serous membranes complicating pneumonia; but two cases in particular have been reported by Thue of pleurisy and pericarditis following pneumonia in which the lymph capillaries have been found to be filled with diplococci, as if injected. Their presence in the blood after death has been amply proved by numerous investigations. In many instances they have been recovered from the blood during life. Lambert, as a rule, found them in all fatal cases twenty-four to forty-eight hours before death. This examination has considerable prognostic value, as nearly all cases in which the pneumococcus is found end fatally. This micrococcus has been shown experimentally to be capable of producing various forms of septicæmia—local phlegmonous inflammations, peritonitis, pleuritis, and meningitis. A further proof of the transmission of this organism by means of the blood is given by Foá and Bordoni-Uffreduzzi in their investigations into intrauterine infection in pneumonia and meningitis. These investigators have demonstrated the presence of the micrococcus lanceolatus in fetal and placental blood and in the uterine sinuses in maternal pneumonia. There being no question, therefore, as to the possibility of the conveyance of the infective agent by means of the blood and the lymph to all parts of the body, we need not wonder at the multiplicity of the affections complicating a pneumonia, which are caused by this micrococcus; and not only the secondary, but also the primary diseases, as of the brain and meninges, may be explained in the same way. Knowing that the saliva and nasal secretions under normal conditions

so frequently afford a resting place for the micrococci, we have only to assume the production of a suitable culture medium for these parasites in the body, brought about by an abnormal condition of the mucous membranes from exposure to cold, or a reduction of the vital resisting power of the tissue cells in any of the internal organs, caused by disease, traumatism, excesses of various kinds, etc., to comprehend readily how an individual may become infected with pneumococci, either primarily affecting the lungs and secondarily other organs in the body, or primarily attacking the middle ear, the pericardial sac, the pleura, the serous cavities of the brain, etc.

Presence in Inflammatory Processes Not Secondary to Pneumonia.—It is now known that the pneumococcus may infect and excite disease in many tissues of the body independent of any preliminary localization in the lung. As a rule, these processes are acute and usually run a shorter and more favorable course than similar inflammations due to the streptococci.

The most frequent primary lesions excited by the pneumococcus after lobar pneumonia, bronchopneumonia, and bronchitis are probably meningitis, otitis media, endocarditis, pericarditis, rhinitis, tonsillitis, conjunctivitis, and keratitis; septicæmia, arthritis, and osteomyelitis; inflammations of the epididymis, testicles, and Fallopian tubes; peritonitis, etc.

Pneumococcic peritonitis is not so very infrequent. The exudate is usually seropurulent.

Conjunctivitis due to pneumococci frequently occurs in epidemic form and is frequently associated with a rhinitis.

From statistics collected by Netter the following percentages of diseases were caused by the pneumococcus:

Pneumonia	65.9 per cent.	in adults.
Bronchopneumonia	15.8 "	"
Meningitis	13.0 "	"
Empyema	8.5 "	"
Otitis media	2.4 "	"
Endocarditis	1.2 "	"

In 46 consecutive pneumococcus infections in children there were:

Otitis media	29 cases.
Bronchopneumonia	12 "
Meningitis	2 "
Pneumonia	1 "
Pleurisy	1 "
Pericarditis	1 "

The pneumococcus and streptococcus are the two most frequent organisms found in otitis media. The cases due to the pneumococcus are apt to run the shorter course, but have a tendency to spread to the meninges and cause a meningitis. The pneumococci may also find their way into the blood current.

In bronchitis the pneumococcus is frequently met with alone or in

combination with the streptococcus, the influenza bacillus, or other bacteria.

In certain epidemics pneumococcic bronchitis and pneumonia simulate influenza very closely and cannot be differentiated except by bacteriological examinations.

Primary pneumococcic pleurisy is frequent in children; it is very often purulent, but may be serous or serofibrinous. Its prognosis is better than that in cases due to other organisms. Frequently we have streptococci and staphylococci associated with the pneumococci.

Varieties of the Pneumococcus.—As among all other micro-organisms minutely studied, different strains of pneumococci show quite a wide range of variation in morphology and virulence. Some of the variations are so marked and so constant that they make it necessary to recognize several distinct varieties of the pneumococcus, and to class as pneumococci certain varieties which have before this been classed as streptococci—*e. g.*, the so-called *streptococcus mucosus capsulatus* (*streptococcus mucosus* Schottmüller), when first isolated from pneumonic exudate or elsewhere, and planted on artificial culture media containing serum, grows as a rounded coccus with a small dense distinct capsule, principally in short or medium chains; it produces a large amount of mucus-like zooglia, forming very large spreading colonies; it promptly coagulates fluid serum media containing inulin. It is also very virulent for mice, but only moderately virulent for rabbits. After a number of culture generations on ordinary nutrient agar it apparently loses most of these characteristics. It then grows in small colonies principally as naked diplococci which may be elongated and pointed, produces no zooglia, and loses most of its virulence for mice and rabbits. It still coagulates inulin serum media, and when transferred to serum media regains its former morphological characteristics. For these reasons we consider this organism a distinct variety of the pneumococcus. This variety of pneumococcus has been isolated by us from the lungs after death following lobar pneumonia, out of twenty consecutive autopsies, as the only organism present twice, and with another variety of pneumococcus once. Together with other varieties it was isolated from four out of twenty specimens of pneumonic sputum, and from sixty specimens of normal throat secretion five times.

Another group of pneumococci quite constantly produces large forms and large capsules. Still another group produces principally small forms and small capsules. Another group might be made of morphologically typical pneumococci which do not coagulate inulin serum media.

Immunity.—Early in the history of this organism experiments were begun for the production of immunity in animals by means of preventive inoculations. Later it was found that after successive injections of gradually increasing doses of virulent pneumococci into certain animals (horse, sheep, goat, rabbit), a serum of some protective and curative power in experimental animals was obtained. The mode of action of this serum is still the subject of study. According to Wright, Neufeld,

and others, its activity is due to the presence of certain substances called opsonins (Wright), or bacteriotropic substances (Neufeld), which act on the bacteria in such a way as to prepare them for ingestion by the phagocytes.

Agglutination Reactions.—It has been shown that the specific serum obtained by the above method may contain a considerable quantity of agglutinating substances for the strain of pneumococcus inoculated and for certain other strains, but not for all. In the case of the *pneumococcus mucosus* (*streptococcus mucosus* Schottmüller) we have found that all of the strains tested by us were agglutinated in high dilutions in the serum obtained after the inoculation of one strain.

Therapeutic Experiments.—The number of cases reported in which the blood-serum of animals artificially immunized against pneumonic infection has been used for the treatment of the disease in human beings, although numerous has not led to the formation of a definite opinion as to the final value of this as a therapeutic agent. In the cases we have observed there has been in some a slight immediate lowering of the temperature; in others no apparent change. As a rule, the cases did rather better than was expected, but certainly no striking curative effects were apparent. The cases did not develop pneumococcus blood infection, and it seems probable that the serum may be able to prevent a general infection from taking place from the diseased lung, even though it may fail to influence the local process. It has also been shown that these injections of antipneumococcic serum are practically harmless. In pneumococcus septicæmia no marked results have been seen. The majority who received the injections, as well as those not receiving them, died.

The Pneumobacillus of Friedländer.

This bacillus discovered by Friedländer (1883) is now known to occur frequently as a mixed infection in cases of phthisis, fibrinous pneumonia, and in rare instances as the only exciting factor in pneumonia. It is also not infrequently found in the mucous membranes of the mouth and air passages of healthy individuals.

Morphology.—Short bacilli with rounded ends, often resembling micrococci, especially in recent cultures; commonly united in pairs or in chains of four, and, under certain circumstances, surrounded by a transparent capsule. This capsule is not seen in preparations made from artificial culture media, but is visible in well-stained preparations from the blood of an inoculated animal.

Friedländer's bacillus *stains* readily with the aniline colors, but is not stained by Gram's method.

Biology.—An aërobic, non-motile, non-liquefying bacillus; also facultative anaërobic; does not form spores. In *gelatin stick cultures* it presents the "nail-shaped" growth first described by Friedländer, which is not, however, peculiar to this bacillus. Gas bubbles occasionally develop in gelatin, and in old cultures the gelatin acquires a distinct

brownish coloration. This latter characteristic distinguishes the growth of this bacillus from that of the bacillus aërogenes, which is otherwise very similar to it morphologically and culturally. On *gelatin plates* colonies appear at the end of twenty-four hours as small white spheres, which rapidly increase in size. These colonies, when examined by a low-power lens, present a somewhat irregular outline and a slightly granular appearance. The growth on *agar* is in quite large and moist grayish colonies. On *blood serum* abundant, grayish-white, viscid masses are developed. The growth on *potato* is luxuriant—a thick, yellowish-white, glistening layer rapidly covering the entire surface. *Milk* is not coagulated. *Indol* is produced in bouillon or peptone solutions. Milk-sugar and glucose are fermented. Growth occurs at 16° to 20° C., but is more rapid at 37° C.

Pathogenesis.—Friedländer's bacillus is pathogenic for mice and guinea-pigs, less so for dogs, and rabbits are apparently immune. On autopsy after death due to inoculation into the lungs, the pleural cavities are found to contain a seropurulent fluid, the lungs are intensely congested, and in places show limited areas of red hepatization; the spleen is considerably enlarged, and bacilli are present in the lungs, the pleuritic fluid, and the blood.

Friedländer's bacillus has been found in man, not only in patients suffering from croupous pneumonia and other respiratory diseases, but also in healthy individuals, and in the outside world. Netter observed it in 4.5 per cent. of the cases examined by him in the saliva of healthy individuals, and Pansini in cases of pulmonary tuberculosis in the sputum. Friedländer believed that the bacillus described by him was the specific cause of croupous pneumonia; but in 129 cases examined by Weichselbaum this bacillus was found in only 9; of 70 cases examined by Wolf only 3 showed the presence of Friedländer's pneumobacillus. It is evident, therefore, that though this micro-organism may be concerned in the production of certain forms of the disease, it is not the specific cause of croupous pneumonia. The cases which are due primarily to the pneumobacillus are distinguished, according to Weichselbaum and Netter, by their peculiarly malignant type and by the viscidity of the exudate produced. This bacillus is also probably concerned, primarily or secondarily, under certain circumstances, in the production of pleurisy, abscess of the lungs, pericarditis, endocarditis, otitis media, and meningitis, in all of which diseases it has at times been found to be present.

CHAPTER XXVI.

MENINGOCOCCUS OR DIPLOCOCCUS (MICROCOCCUS) INTRACELLULARIS MENINGITIDIS, AND THE RELATION OF IT AND OF OTHER BACTERIA TO MENINGITIS.

In the description of the diplococcus of pneumonia reference was made to this organism as the most frequent cause of isolated cases of meningitis, especially when it complicated pneumonia. In 1887 Weichselbaum discovered another micrococcus in the exudate of cerebrospinal meningitis in six cases, two of which were not complicated by pneumonia. He obtained it in pure cultures, studied its characteristics, and showed that this organism was clearly distinguishable from the micrococcus lanceolatus, and especially by its usual presence in the interior of pus-cells, on which account he called it *diplococcus intracellularis meningitidis.* The frequency of the occurrence of this diplococcus in meningitis and its almost complete restriction to this disease affords sufficient evidence for the assumption that it was concerned in its production. In 1895 Jaeger and Scheurer believed they found it in the nasal secretions of eighteen living persons suffering from this disease during an epidemic.

It seems very probable that in most cases of primary meningitis it is from the mucous membrane of the nasal cavities and the sinuses opening out from them that both the diplococcus of pneumonia and the micrococcus intracellularis find their way through the lymph channels to the meninges. The former we know to be almost constantly present in the nasal cavities, and the latter we have reason to believe is not infrequently there. The prevalence of epidemics in winter and spring, a time favorable to influenza and pneumonia, also suggests the respiratory tract as the source of the infection and the place where an increase in virulence takes place. We do not as yet know why meningitis follows in some persons and not in others after infection of the mucous membranes.

The meningococcus dies readily when dried, so that we seldom inhale it except in rooms occupied by those infected. Such persons and things recently soiled by their nasal secretion are especially dangerous.

Morphology.—This organism occurs as biscuit-shaped micrococci, usually united in pairs, but also in groups of four and in small masses; sometimes solitary and small degenerated forms are found. Cultures resemble strongly those of gonococci (see Fig. 111). In cultures more than twenty-four hours old larger and smaller forms occur and some which stain poorly. These are involution forms. In the exudation, like the gonococcus, to which it bears a close resemblance in form and arrangement, it is distinguished by its presence, as a rule,

within the polynuclear leukocytes. It never appears within the nucleus and rarely within other cells (Fig. 110).

Staining.—It *stains* with all the ordinary aniline colors, but best with Loeffler's methylene blue. It is, as a rule, readily decolorized by Gram's solution. Some organisms in many cultures are more resistant than others, but none are definitely Gram positive. It is almost certain that the positive cocci which have been described as meningococci are really contaminating organisms.

Biology.—It grows between 25° and 40° C., best at about 37.5° C., and its development is usually scanty on the surface of nutrient agar, but sometimes a few colonies grow quite vigorously. Now and then cultures grow at 23° C. or slightly less. It grows scarcely at all in bouillon, and scantily in bouillon plus one-third blood serum. It develops comparatively well on Loeffler's blood-serum medium as used for diphtheria cultures, and on blood serum or ascitic-fluid agar.

FIG. 110

Diplococcus intracellularis meningitidis. × 1100 diameters.

Of the usual sugars the meningococcus ferments dextrose only and even this not sufficiently to coagulate the serum media. It grows on 5 per cent. glycerin agar as well as on plain agar.

When grown on nutrient agar or glycerin agar, a tolerably good growth develops at the end of forty-eight hours in the incubator. This appears as a flat layer of colonies, about one-eighth of an inch in diameter, grayish-white in color, finely granular, rather viscid, and non-confluent unless very close together. On Loeffler's blood serum the growth forms round, whitish, shining, viscid-looking colonies, with smooth and sharply-defined outlines; these may attain diameters of one-eighth to one-sixteenth of an inch in twenty-four hours. The colonies tend to become confluent and do not liquefy the serum. From the spinal fluid in acute cases, where the organisms are apt to be more abundant, a great many minute colonies may develop instead of a few larger ones. On agar plates the deep-lying colonies are almost invisible to the naked eye; somewhat magnified they appear finely granular, with a

dentated border. On the surface they are larger, appearing as pale disks, almost transparent at the edges, but more compact toward the centres, which are yellowish-gray in color. On blood agar or serum agar the growth is much more luxuriant than on plain agar and larger than the gonococcus. Not infrequently no growth is obtained when the cerebrospinal fluid containing the diplococci is placed on plain agar, and in rare instances no growth appears when serum agar is used. Cultivated in artificial media while it often lives for weeks, it may die within four days, and requires, therefore, to be transplanted to fresh material at short intervals—at least every two days.

Resistance.—It is readily killed by heat, sunlight, and drying.

Pathogenesis.—This organism does not show marked pathogenic power for adult animals. It is most pathogenic for mice and guinea-pigs, less so for rabbits and dogs. Subcutaneous injections in animals when large cause death; intrapleural or intraperitoneal inoculations in mice and guinea-pigs, when given in large doses ($\frac{1}{10}$ to $\frac{1}{2}$ of a blood-serum culture), are usually fatal. Intravenous injections in rabbits have caused the death of the animal, but no increase of diplococci in the blood or characteristic pathological changes have been found as a result of the injections.

When mice are inoculated into the pleural or peritoneal cavities they usually fall sick and die within thirty-six to forty-eight hours, showing slight fibrinopurulent exudation. In the blood and enlarged spleen diplococci are found in small numbers and mostly free; in the pleuritic exudation they are present in considerable quantities, less so in the peritoneal fluid, but then occurring in the interior of pus cells.

Certain experiments made by Weichselbaum on dogs, though not entirely successful, are interesting as showing the similarity of the disease produced in them artificially with meningitis as occurring in man. The three dogs, trephined and inoculated subdurally with 0.5 to 2 c.c. of a fresh culture, all died: No. 1 within twelve hours, No. 2 in three days, and No. 3 in twelve days. In Nos. 1 and 2 there were found hyperæmia of the meninges, with inflammatory softening of the brain at the point of inoculation, which on nearer inspection proved to be a true encephalitic process. In dog No. 2, in which the disease was of longer duration, these changes were the most pronounced. Numerous diplococci were observed in the sections removed, for the most part free, but some few within the pus cells. In dog No. 3, in which the disease lasted twelve days, a thick, reddish, purulent liquid was found between the dura mater and the brain at the point of inoculation; in the brain itself an abscess had formed, about the size of a hazel-nut, filled with tough, yellow pus, while the abscess walls consisted of softened brain substance infiltrated with numerous hemorrhagic deposits. The ventricles on that side contained a cloudy, reddish fluid, with flocks of pus; but no diplococci could be demonstrated in the blood or exudations. In our experience injection of a recent culture into the spinal canal of very young puppies is regularly followed by the results noted by Weichselbaum. Such effects are not observed in older dogs.

Presence in the Nasal Cavity of the Sick and the Well.—In 1 of his 6 cases Weichselbaum succeeded in obtaining in pure culture diplococci from the nasal secretion. Scheurer, in his 18 cases, found the diplococci in the nasal secretions of all of them during life. In 50 healthy individuals examined they were found in the nasal secretions of only two, one being a man suffering at the time from a severe cold. This man, it is interesting to note, had been engaged in disinfecting a room which had previously been occupied by a patient with cerebrospinal meningitis. Lately, there has been a tendency to throw doubt on these findings, but from our experience in the recent epidemic in New York, one can state that the meningococci are usually present in great numbers in the nose and nasopharynx in most cases of meningitis during the first twelve days of illness. After the fourteenth day they cannot usually be found. In one case Goodwin of our laboratory obtained them on the sixty-seventh day. She also found them in five persons out of sixty tested who had been in close contact with the sick, and in two of fifty medical students.

Complicating Infections.—Occasionally we find secondary to the cerebrospinal meningitis, and due to the micrococcus, inflammations of nasal cavities and their accessory sinuses, also catarrhal inflammations of the middle ear, acute bronchitis, and pneumonia. The absolute determination of the identity of the micrococcus found in these conditions has not been established, so that the above complications can only be considered as probably due to this organism.

Except in cases of meningitis the micrococcus has been absolutely identified only in cases of rhinitis. Several observers believe they have found it in the diseases mentioned above as occasionally complicating meningitis.

Meningococci in the Blood.—Elser in forty cases examined during the early days of the disease found them in ten.

Agglutination Characteristics.—Meningococci are agglutinated in dilutions of the blood serum of animals immunized to any true culture. As a rule dilutions higher than 1 : 40 do not give reactions. In the second and third weeks of disease, agglutination in 1 : 10 or higher dilutions of serum may be obtained.

Serum Treatment.—The use of specific or other sera has not proven of value.

Bacteriological Diagnosis.—By means of lumbar puncture, fluid can readily be obtained from the spinal canal without danger. The skin must be thoroughly cleansed and the needle aseptic. The fluid should be placed in a sterile conical glass to settle. The sediment should be used to make smears to examine (1) for pus cells, (2) for tubercle bacilli, and (3) for other organisms. By Gram's stain we are able to separate the three Gram-positive organisms met with in meningitis (pneumococcus, streptococcus, and staphylococcus) from the others. Of importance also is the point that the micrococcus intracellularis is usually inside the leukocytes in the form of diplococci of varying size, of coffee-bean shape, or of tetracocci, while the pneumococcus is

frequently outside the cells and is usually spherical or lancet-shaped and frequently occurs in short chains. Sometimes the bacteria are present in very small numbers, and then many smears must be looked through before a probable diagnosis can be made. In all cases absolute certainty can only be obtained through cultures. Here plain nutrient agar, serum agar, and blood-agar plates should be made, and, if desired, tubes also. When considerable quantities are inoculated upon these media and meningococci are present, as a rule, a greater or less number of colonies having the characteristics already described will develop. The value, clinically, of the examination is that about 40 per cent. of the cases due to this coccus recover, while almost all of those due to the pneumococcus and streptococcus die.

In many cases there are very few diplococci present in the spinal fluid, so that a failure to find them in a microscopic examination should not be taken to prove that the disease was not due to this organism. For cultures a considerable amount of fluid must be used, for we have found, as described by Councilman and others, that there may be very few living diplococci even in 1 c.c. of fluid.

To obtain the fluid the patient should lie on the right side with the knees drawn up and the left shoulder depressed. The skin of the patient's back, the hands of the operator, and the large antitoxin syringe should be sterile. The needle should be 4 cm. in length, with a diameter of 1 mm. for children, and longer for adults.

The puncture is generally made between the third and fourth lumbar vertebræ. The thumb of the left hand is pressed between the spinous processes, and the point of the needle is entered in the median line or a little to the right of it, and on a level with the thumb-nail, and directed slightly upward and inward toward the median line. At a depth of 3 or 4 cm. in children and 7 or 8 cm. in adults the needle enters the subarachnoid space, and on withdrawing the obturator the fluid flows out in drops or in a stream. If the needle meets a bony obstruction withdraw and thrust again rather than make lateral movements. Any blood obscures the microscopic examination. The fluid is allowed to drop into absolutely sterile test-tubes or vials with sterile stoppers. From 5 to 15 c.c. should be withdrawn. No ill effects have been observed from the operations. On the contrary the relief of pressure frequently produces beneficial results.

Organisms Exciting Meningitis.—1. The *pneumococcus*. This diplococcus is one of the most frequent exciters of meningitis, not only when it is a primary disease, but also when it is secondary to a pneumonia, otitis, etc.

2. The *streptococcus pyogenes* and the *staphylococcus pyogenes*. Meningitis due to these organisms is almost always secondary to some other infection, such as otitis, tonsillitis, erysipelas, endocarditis, suppurating wounds of scalp and skull, etc.

3. *The bacillus influenzæ*. Numerous doubtful reports have been published of the presence of influenza bacilli in the meningeal exudate. Those that are reliable state in almost every instance that the menin-

gitis is secondary to infection of the lungs, bronchi, the nasal cavities or their accessary sinuses.

4. The *colon bacillus*, the *typhoid bacillus*, that of *bubonic plague* and of *glanders*, all may cause a complicating purulent meningitis.

5. In isolated cases of meningitis complicating otitis media and other infections, other bacteria, such as the *micrococcus tetragenus*, the *bacillus pyocyaneus*, etc., may be found

Micrococcus Catarrhalis (R. Pfeiffer).

Micrococci somewhat resembling meningococci are found in the mucous membranes of the respiratory tract. They are believed at times to excite catarrhal inflammation of the mucous membranes. These are at present included under the designation of micrococcus catarrhalis.

Microscopic Appearance.—They usually occur in pairs, sometimes in fours; never in chains. The cocci are coffee-bean in shape and slightly larger than the gonococcus, and are negative to Gram's stain.

The micrococci are not motile and produce no spores.

Cultivation.—They grow between 20° and 40° C., best at 37° C. and less rapidly at somewhat lower temperatures. They develop on ordinary nutrient agar as grayish-white or yellowish-white, circular colonies of the size of meningococci. The borders of the colonies are irregular and abrupt as though gouged out. They have a mortar-like consistency. On serum-agar media the growth is more luxuriant. Gelatin is not liquefied. Bouillon is clouded, often with the development of a pellicle. Milk is not coagulated, but dextrose serum media may be. Gas is not produced.

Location of Organisms.—In the secretion of normal mucous membranes they are occasionally present. In certain diseased conditions of the mucous membranes they may be abundant.

Pathogenic Effects in Animals.—For white mice, guinea-pigs, and rabbits, some cultures are as pathogenic as meningococci, while others are less so.

Differential Points Separating them from the Meningococci.—These organisms have undoubtedly been at times confused. Some assert that the meningococci grow only above 25° C. Many cord cultures of meningococci grow below this point. Some assert that the meningococci will not grow on 5 per cent. glycerin agar. Many undoubted cultures do. The probability is that the organisms described by different writers as micrococcus catarrhalis were not all the same variety, and some of them were meningococci.

CHAPTER XXVII.

THE GONOCOCCUS OR MICROCOCCUS GONORRHŒÆ—THE DUCREY BACILLUS OF SOFT CHANCRE.

THE period at which gonorrhœa began to afflict man is unknown. The earliest records make mention of it. Except for a period after the fifteenth century it was generally recognized as a communicable disease and laws were made to control its spread. The differentiation between the lighter forms of gonorrhœa and some other inflammations of the mucous membranes was, however, almost impossible until the discovery of the specific micro-organism by Neisser, in 1879.

The organism was first observed in gonorrhœal discharges, and described by him under the name of "gonococcus;" but though several attempted to discover a medium upon which it might be cultivated, it was reserved for Bumm, in 1885, to obtain it in pure culture upon coagulated human-blood serum, and then after cultivating it for many generations to prove its infective virulence by inoculation into man. The researches of Neisser and Bumm established beyond doubt that this organism is the specific cause of gonorrhœa in man.

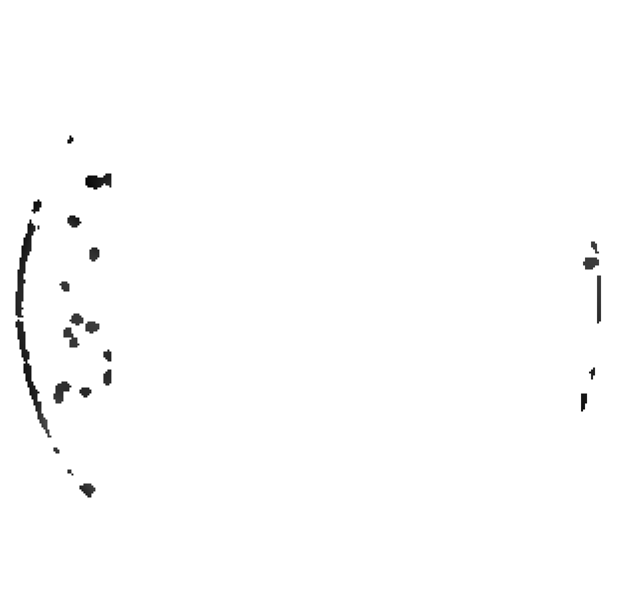
Microscopic Appearance.—Micrococci, occurring mostly in the form of diplococci. The bodies of the diplococci are elongated, and, as shown in stained preparations, have an unstained division or interspace between two flattened surfaces facing one another, which give them their characteristic "coffee-bean" or "kidney" shape. The older cocci lengthen, then become constricted in their middle portion, and finally divide, making new pairs (Fig. 111). The diameter of an associated pair of cells varies according to their stage of development from 0.8μ to 1.6μ in the long diameter—average about 1.25μ—by 0.6μ to 0.8μ in the cross diameter.

Intracellular Position of Gonococci.—In gonorrhœa, during the earliest stages before the discharge becomes purulent, the gonococci are found mostly free in the serum or plastered upon the epithelium cells, but later almost entirely in small, irregular groups in or upon the pus cells, and always extranuclear. With the disappearance of the pus formation more free gonococci appear. Discharge expressed from the urethra usually contains more free organisms than the natural flow. Gonococci are sometimes irregular in shape or granular in appearance, involution forms, found particularly in older cultures and in chronic urethritis of long standing. Single pus cells sometimes contain as many as one hundred gonococci and seem to be almost bursting and yet show but slight signs of injury. There is still discussion as to whether the gonococci actively invade the pus cells or only are taken

up by them. There is no evidence that the gonococci are destroyed by the pus cells (Fig. 112).

Staining.—The gonococcus *stains* readily with the basic aniline colors. Loeffler's solution of methylene blue is one of the best staining agents for demonstrating its presence in pus, for, while staining the gonococci deeply, it leaves the cell protoplasm but faintly stained. Fuchsin is apt to overstain the cell substance. Beautiful double-stained preparations may be made from gonorrhœal pus by treating cover-glass smears with methylene blue and eosin. Numerous methods for double staining have been employed, with the object of making a few gonococci more conspicuous. None of them have any specific characteristics such as the Gram stain. It is now established that gonococci from fresh cultures and from recent gonorrhœal infections are, when properly treated by Gram's method, quickly and surely robbed of their color. The removal of the stain from gonococci in old flakes

FIG. 111



Smear from pure culture of gonococcus on agar. × 1100 diameters. (Heiman.)

FIG. 112

Gonococcus in pus cells. × 1100 diameters.

and threads from chronic cases is not so certain. This difference is mostly due to the fact that equally uniform specimens cannot be prepared. The decolorized gonococci are stained by dipping the films for a few seconds into a 1 : 10 dilution of carbol-fuchsin, or a solution of bismarck brown This staining should be for as short a time as suffices to stain the decolorized organisms. This method of staining cannot be depended upon alone to absolutely distinguish the gonococcus from all other diplococci found in the urethra and vulvovaginal tract, for, especially in the female, other diplococci are occasionally found which are also not stained by Gram's method. It serves, however, to distinguish this micrococcus from the common pyogenic cocci, which retain their color when treated in the same way, and in the male urethra it is practically certain, as no organism has been found in that location which in morphology and staining is identical with the gonococcus. It is certainly the most distinctive characteristic of the staining properties of the gonococcus, and it is a test

that should never be neglected in differentiating this organism from others which are morphologically similar.

Biology.—Grows best at blood temperature; the limits being roughly 25° and 40° C. It is a facultative anaërobe. It is not motile and produces no spores.

Culture Media.—The gonococcus requires for its best growth the addition to nutrient agar of a small percentage of blood serum or some equivalent. The following media have proven of value:

1. Human blood from the sterilized finger streaked on common nutrient agar.
2. Human-blood serum, 1 part added to and mixed with 2 parts melted 5 per cent. glycerin nutrient, 1.5 per cent. agar having a temperature of 55° to 60° C. The whole after mixing being poured into a Petri dish or cooled slanted in a tube. The same proportions of nutrient broth and serum make a suitable fluid media.
3. Human ascitic, pleuritic or cystic fluid in same proportions as blood serum. One per cent. glucose may be added.
4. Swine serum nutrose media. Wasserman strongly recommends this mixture. (See under Media.) In our hands it has given good results.
5. Nutrient or 5 per cent. glycerin agar. When considerable pus is streaked on simple agar media a good growth of gonococci is usually obtained. After continued cultivation gonococci cultures frequently grow on media containing no serum. Some strains grow on ordinary glycerin or glucose nutrient agar from the start.

Viability.—Cultures frequently die in forty-eight to seventy-two hours when kept at room temperature. In the ice-box they may live for several weeks. Most cultures require the serum media for the later cultures,

Appearance of Colonies.—A delicate growth is characteristic. At the end of twenty-four hours there will have developed translucent, very finely granular colonies, with scalloped margin. The margin is sometimes scarcely to be differentiated from the culture medium. In color they are grayish-white, with a tinge of yellow. The texture is finely granular at the periphery, presenting punctated spots of higher refraction in and around the centre of yellowish color (Fig. 113).

Surface Streak Culture.—Translucent grayish-white growth, with rather thick edges.

Resistance.—The gonococcus has but little resistant power against outside influences. It is killed by weak disinfecting solutions and by desiccation in thin layers. In comparatively thick layers, however, as when gonorrhœal pus is smeared on linen, it has lived for forty-nine days, and dried on glass for twenty-nine days (Heiman). It is killed at a temperature over 42° C.

Occurrence of Gonococci.—Outside of the human body or material carried from it gonococci have not been found.

Pathogenesis.—Non-transmissible to all animals. Both the living and dead gonococci contain toxic substances which cause death or injury when injected in large quantities.

Though animal inoculations are thus followed by negative results, the etiological relation of the gonococcus to human gonorrhœa has been demonstrated beyond question by the infection of a number of healthy men with the disease by the inoculation of pure cultures of the micro-organism.

Toxins.—In the gonococcus cells substances are present which are toxic after heating and contact with alcohol. Injected in considerable amounts into rabbits they cause infiltration and often necrosis. Applied to the urethral mucous membrane there is produced an inflammation of short duration. In gonorrhœa the secretion is believed to be due to these intracellular toxins. Repeated injections give no appreciable immunity. The filtrate of recent gonococcus cultures contains little or no appreciable toxin. The typical incubation and symptoms of the disease resulted in all cases in the subjects experimented on.

Fig. 113

Colonies of gonococci on pleuritic fluid agar. (Heiman.)

Disease Conditions Excited by Gonococci.—Affections due to this organism are usually restricted to the mucous membranes of the urethra, prostate, neck of bladder, cervix uteri, vagina, and conjunctiva. The conjunctival, vaginal, and rectal mucous membranes are much more sensitive in early childhood than in later life. Besides these tissues it has been proven that many others may be infected, so that we now know that to the gonococci are due many cases of endometritis, metritis, salpingitis, oöphoritis, peritonitis, proctitis, cystitis, epididymitis, and arthritis. Abscesses of considerable size, periostitis, and otitis are occasionally due to the gonococcus.

Endocarditis and Septicæmia.—Cases of gonococcus endocarditis and septicæmia are not infrequent. Gonococcus septicæmia may occur in connection with other localizations or alone. Nearly every year one or two of these cases is met with in every general hospital. In a considerable number of cases where gonococci are obtained from the blood the patients recover. The fever is sometimes typhoid-like in character.

Pavement epithelium is more difficult to infect than cylindrical. The gonococcus gradually penetrates the epithelial layer and produces inflammation of the connective tissue.

Immunity.—Immunity in man after recovery from infection seems to be only slight in amount and for a short period if present at all. It is known that the urethra in man or cervix uteri in woman may contain gonococci which lie dormant and may be innocuous in that person for years, but which may at any time excite an acute gonorrhœa in another individual or, under stimulating conditions, in the one carrying the infection.

Duration of Infections and of Contagious Period.—There is no limit to the time during which a man or woman may remain infected with gonococci and infect others. We have had one case under observation where twenty years had elapsed since exposure to infection, and yet the gonococci were still abundant. It is now well established that most of the inflammations of the female genital tract are due to gonococci, and the majority of such infections are produced in innocent women by their husbands who are suffering from latent gonorrhœa.

Bacteriological Diagnosis of Gonorrhœa.—In view of the fact that several non-gonorrhœal forms of urethritis exist, and also that micrococci morphologically similar to the gonococcus Neisser are often found in the normal vulvovaginal tract, it becomes a matter of great importance to be able to detect gonococci when present, and to differentiate these from the non-specific organisms. Besides this, the gonococci which occur in old cultures and in chronic urethritis of long standing sometimes take on a very diversified appearance. From a medicolegal and social standpoint, therefore, the differential diagnosis of the gonococcus has in certain cases a very practical significance.

There are two methods of differential diagnosis now available—the microscopic and the cultural. Animal inoculations are of no value, as animals are not susceptible, and, of course, human inoculations are generally impossible. In the microscopic diagnosis it should be borne in mind that after the acute serous stage has passed, the specific gonococci in *carefully made* preparations are always found largely within the pus cells. Diplococci morphologically similar to gonococci occurring in other portions of the field and outside of the pus cells should not be considered specific by this test only. It should also be remembered that the gonococci are decolorized by Gram's method, while other similar micrococci which occur in the urethra are, as a rule, at least not so decolorized. Organisms having these characteristics can for all practical purposes be considered as certainly gonococci if obtained from the urethra. From the vulvovaginal tract the certainty is not so great, since other diplococci are occasionally found in gonorrhœal pus from the vulvovaginal tract, and very rarely, also, from the urethra, which do not stain by this method; here cultures should also be made. Cover-glass preparations from subacute or chronic cases should be examined, if possible, with a microscope provided with a mechanical stage, and films should always be stained by

both Loeffler's methylene-blue solution and by Gram's method, and the examination repeated on three consecutive days. Should these specimens prove negative, to exclude any possible doubt in the matter, cultures should then be made on human ascitic fluid or serum agar, poured in dishes; also, if with negative results, on three consecutive days. Heiman, who has paid much attention to gonococcus examinations, obtains his material by the following method: in chronic urethritis he allows the patient to void his urine either immediately into two sterilized centrifugal tubes or first into two sterile bottles. The first tube will contain threads of the anterior urethra; the second tube will be likely to contain secretion from the posterior urethra and from the prostate gland if, while urinating, the patient's prostate be pressed upon with the finger. Tubes containing such urine are placed in the centrifuge and whirled for three minutes at twelve hundred or more revolutions per minute; the threads are thrown down. The centrifuged sediment will be found to contain most of the bacteria present, epithelial cells, and, at times, spermatozoa. Normal urine on being centrifuged at this velocity will be found at times to show a slight turbidity at the bottom of the tube. This will be found, on microscopic examination, to consist of epithelial cells, a few leukocytes, and some bacteria.

The careful examination of gonorrhœal threads stained by Gram's method is a very tedious affair, as in every instance no less than three cover-glass preparations should be looked over before the absence of the gonococcus is considered probable. It would require many hours upon each and every specimen, especially if the gonococci are present in very small number, before a reliable and conscientious opinion could be rendered. If, after all, a negative opinion is ventured, we still are under the necessity of proving that because the threads which we fished out for the cover-glass examination were free from gonococci the remaining ones were also. For this reason the culture medium is more sensitive for bacteria than is the cover-glass, for we are able to plant each and every thread of the sediment in the centrifugal tube. Results on culture media are only reliable when obtained by thoroughly trained bacteriologists with suitable media and methods. Fürbringer, in his work, mentions the fact that in certain cases the absence of the gonococcus in many examinations of cover-glass preparations is not a positive proof that the gonococcus is not present. The culture methods, of course, presuppose that one has the facilities and knowledge to carry them out successfully, otherwise the microscopic methods are to be used alone.

In acute cases where the pus is abundant the specimen for examination may be collected, when the patient is before one, by passing a sterilized platinum-wire loop as far up into the urethra as possible and withdrawing some of the secretion.

Occurrence in Cultures from Chronic Urethritis.—Heiman in 61 cases found the gonococcus in 14 by cultures and in 13 by smears. The following results were obtained by other observers by cover-glass preparations: Goll, according to his elaborate article, examined 1046 cases

of chronic urethritis varying in duration between four weeks to six years or more, finding gonococci in 178 cases, the remainder giving negative results. Neisser, out of 143 cases, varying in duration between two months and eight years, found gonococci in 80 cases.

BACTERIA RESEMBLING GONOCOCCI.

Bumm described a number of micrococci which resembled gonococci in form and staining. These assume importance largely because they may be confused with the gonococcus. They occur on the conjunctival and vaginal mucous membranes and cause confusion. One of these micro-organisms, the micrococcus catarrhalis (see p. 365), has an importance of its own.

The Micrococcus Melitensis.

This micro-organism was first discovered in a case of Malta fever by Bruce in Malta in 1887. The disease is mostly confined to the shores of the Mediterranean, but cases of it have been observed in Porto Rico and the Philippines. The disease does not seem to be directly transmitted from person to person.

Morphology.—Small rounded or slightly oval organism about 15μ in diameter. It is usually single or in pairs, but in cultures short chains are also met with. Durham has shown that in old cultures bacillary forms occur. Gorham believes he has demonstrated that the coccus has one to four flagella.

Staining.—It stains readily with the aniline dyes and is negative to Gram.

Cultivation.—At 37° C. it grows on nutrient agar and in broth. The colonies are not usually visible until the third day. They appear as small round disks, slightly raised, with a yellowish tint in the centre.

Methods of Diagnosis.—During life the best means of diagnosis is by the agglutination test. The serum from different persons agglutinates in dilutions of from 1:10 to 1:1000. Cultures are readily obtained from the spleen.

The Ducrey Bacillus of Soft Chancre.

This bacillus was first specifically described and obtained in pure culture by Ducrey in 1889.

Morphology.—About 1.5μ long and 0.4μ thick, growing often in chains and in cultures, sometimes twisted together in dense masses.

It *stains* best with carbol-fuchsin, and shows polar staining.

Cultural Characteristics.—The following method of cultivation has given the best results. Two parts agar are liquefied at 50° C. and mixed with one part human, dog, or rabbit blood. The blood from the cut carotid of a rabbit may be allowed to run directly into the agar

tube, to which the pus from the ulcerated bubo is then added in proper proportion, and the whole placed in the incubator at 35° C. The pus may be obtained by puncture and aspiration from the unbroken ulcer, or if the ulcer is already open it is first painted with tincture of iodine and covered with collodion or sterile gauze. After twenty-four to forty-eight hours, some pus having collected under the bandage, inoculations are made from it. The bacillus grows well also in uncoagulated rabbit-blood s rum or in condensation water of blood agar. In twenty-four to forty-eight hours, on the surface of the media, well-developed, shining, grayish colonies, about 1 mm. in diameter, may be observed. The colonies remain separate, but only become numerous after further transplantation. The best results are obtained when the pus is taken close to the walls of the abscess.

Glass smears show isolated bacilli or short parallel chains with distinct polar staining.

After the eleventh generation of the culture, and from all old cultures, on inoculation the characteristic soft chancre is produced in man. According to some observers animals cannot be infected; others claim to have obtained positive results with monkeys and cats.

The organisms are especially characteristic in the water of condensation from blood agar, the bacilli being thinner and shorter, with rounded ends; sometimes long, wavy chains are found. In rabbit-blood serum at 37° C. a slight clouding of the medium is produced and small flakes are formed, consisting of short bacilli or moderately long, curved chains, showing polar staining.

The bacillus lives several weeks on blood agar at 37° C., but it soon dies in cultures on coagulated serum. All other ordinary culture media so far tried have given negative results, and even with the media described development is difficult and often fails entirely.

The chancre bacillus possesses but little resistance to deleterious outside influences. Hence, the various antiseptic bandages, etc., used in treatment of the affection soon bring about recovery by preventing the spread of inoculation chancre.

CHAPTER XXVIII.

BACILLUS PYOCYANEUS (BACILLUS OF GREEN AND OF BLUE PUS)—BACILLUS PROTEUS VULGARIS—GROUP OF MALIGNANT ŒDEMA BACILLI—BACILLUS AËROGENES CAPSULATUS.

Bacillus Pyocyaneus.

THE blue and green coloration which is occasionally found to accompany the purulent discharges from open wounds is usually due to he action of the bacillus pyocyaneus. According to recent investigations this bacillus appears to be very widely distributed. It was first obtained in pure culture by Gessard.

Morphology.—Slender rods from 0.3μ to 1μ broad and from 2μ to 6μ long; frequently united in pairs or in chains of four to six elements; occasionally growing out into long filaments and twisted spirals. The

FIG. 114

Bacillus pyocyaneus. (From Kolle and Wassermann.)

bacillus is actively motile, a single flagellum being attached to one end. Does not form spores. *Stains* with the ordinary aniline colors; does not stain with Gram's solution.

Biology.—An aërobic, liquefying, motile bacillus. Capable also of an anaërobic existence, but then produces no pigment. Grows readily on all artificial culture media at the room temperature, though best at 37° C., and gives to some of them a bright-green color in the presence of oxygen. In *gelatin-plate* cultures the colonies are rapidly developed, imparting to the medium a fluorescent green color; liquefaction begins at the end of two or three days, and by the fifth day the

gelatin is usually all liquefied. The deep colonies, before liquefaction sets in, appear as round, granular masses with scalloped margins, having a yellowish-green color; the surface colonies have a darker green centre, surrounded by a delicate, radiating zone. In *stick cultures in gelatin* liquefaction occurs at first near the surface, in the form of a small funnel, and gradually extends downward; later the liquefied gelatin is separated from the solid part of the medium by a horizontal plane, a greenish-yellow color being imparted to that portion which is in contact with the air. On *agar* a wrinkled, moist, greenish-white layer is developed, while the surrounding medium is bright green; this subsequently becomes darker in color, changing to blue-green or almost black. In *bouillon* the green color is produced, and the growth appears as a delicate, flocculent sediment. *Milk* is coagulated with coincident acid reaction.

There is some difference of opinion in regard to the pigments produced by the bacillus pyocyaneus. Gessard's view is that two pigments are produced by this bacillus—one of a fluorescent green and the other (pyocyanin) of a blue color. Pyocyanin is soluble in chloroform, and may be obtained from pure solution in long, blue needles. The pigment which is thus extracted by chloroform distinguishes the bacillus pyocyaneus from other fluorescing bacteria.

Distribution.—This bacillus is very widely distributed in nature; it is found on the healthy skin of man, in the feces of many animals, in water contaminated by animal or human material, in purulent discharges, and in serous wound secretions.

Pathogenesis.—Its presence in wounds greatly delays the process of repair, and may give rise to a general depression of the vital powers from the absorption of its toxic products. Its pathogenic effects on animals have been carefully studied. It is pathogenic for guinea-pigs and rabbits. Subcutaneous or intraperitoneal injections of 1 c.c. or more of a bouillon culture usually cause the death of the animal in from twenty-four to thirty-six hours. Subcutaneous inoculations produce an extensive inflammatory œdema and purulent infiltration of the tissues; a serofibrinous or purulent peritonitis is induced by the introduction of the bacillus into the peritoneal cavity. The bacilli multiply in the body, and may be found in the serous or purulent fluid in the subcutaneous tissues or abdominal cavity, as well as in the blood and various organs. When smaller quantities are injected subcutaneously the animal usually recovers, only a local inflammatory reaction being set up (abscess), and it is subsequently immune against a second inoculation with doses which would prove fatal to an unprotected animal. It is interesting to note that Bouchard, Charrin, and Guignard have shown that in rabbits which have been inoculated with a culture of the bacillus anthracis a fatal result may be prevented by inoculating the same animal soon after with a pure culture of the bacillus pyocyaneus. Loew and Emmerich have shown that the enzymes produced in the pyocyaneus cultures are capable of destroying many forms of bacteria in the test-tube, and have a slight protecting value in

the body. The pyocyaneus bacillus produces these effects not only through ferments, but by intracellular toxins.

Our knowledge of the pathogenic importance of the bacillus pyocyaneus in human diseases has been much increased by recent investigations. Thus, cases have been reported in which this bacillus has been obtained in pure culture from pus derived from the tympanic cavity in disease of the middle ear, from cases of ophthalmia, and bronchopneumonia. Kruse and Pasquale have found the same microorganism in three cases of idiopathic abscess of the liver, in two of them in immense numbers and in pure culture. Ernst and Schürmayer report the presence of the bacillus pyocyaneus in serous inflammation of the pericardial sac and of the knee-joint. Ehlers gives the history of a disease in two sisters who were attacked simultaneously with fever, albuminuria, and paralysis. It was thought that they would prove to be typhoid fever or meningitis, but on the twelfth day there was an eruption of blisters, from the contents of which the bacillus pyocyaneus was isolated. Jadkewitsch reports the case of a patient suffering from eczema of the lower extremities, in whom three times during a period of ten years there was eruption of boils containing blue pus, with accompanying symptoms of poisoning, emaciation, prostration, diarrhœa, and paresis. Krambals refers to seven cases in which a general pyocyaneus infection occurred, and adds an eighth from his own experience. In this the bacillus pyocyaneus was obtained post-mortem from green pus in the pleural cavity, from serum in the pericardial sac, and from the spleen in pure culture. Schimmelbusch states that a physician injected 0.5 c.c. of sterilized (by heat) culture into his forearm. As a result of this injection, after a few hours he had a slight chill, followed by fever, which at the end of twelve hours reached 38.8° C.; an erysipelatous-like swelling of the forearm occurred, and the glands in the axilla were swollen and painful. Wasserman reports an epidemic of septic infection of the newborn, starting in the umbilicus. In all there were eleven deaths. Lartigau found it in well-water, and in great abundance in the intestinal discharges of a number of cases made ill by drinking the water. It has also been found in a certain number of cases of gastroenteritis, where no special cause of infection could be noted.

We may, therefore, conclude from these facts that the bacillus pyocyaneus, although ordinarily but slightly pathogenic for man, may under certain conditions become a dangerous source of infection. Children would seem to be particularly susceptible.

The differential diagnosis of the pyocyaneus from other fluorescing bacteria is easy enough as long as it retains its pigment-producing property. When an agar culture is agitated with chloroform a blue coloration demonstrates the presence of this bacillus. When the pyocyanin is no longer formed, however, the diagnosis is by no means easy, particularly when the pathogenic properties are also gone.

Bacillus Proteus Vulgaris.

This bacillus, which is one of the most common and widely distributed putrefactive bacteria, was discovered by Hauser (1885) along with other species of proteus in putrefying substances. These bacteria were formerly included under the name "bacterium termo" by previous observers, who applied this name to any minute motile bacilli found in putrefying infusions.

Morphology.—Bacilli varying greatly in size; most commonly occurring 0.6μ broad and 1.2μ long, but shorter and longer forms may also be seen, even growing out into flexible filaments which are sometimes more or less wavy or twisted like braids of hair.

The bacillus does not form spores, and *stains* readily with fuchsin or gentian violet.

Biology.—An aërobic, facultative anaërobic, liquefying, motile bacillus. Grows rapidly in the usual culture media at the room temperature.

GROWTH ON GELATIN—The growth upon *gelatin plates* containing 5 per cent. of gelatin is very characteristic. At the end of ten or twelve hours at room temperature small, round depressions in the gelatin are observed, which contain liquefied gelatin and a whitish mass consisting of bacilli in the centre. Under a low-power lens these depressions are seen to be surrounded by a radiating zone composed of two or more layers, outside of which is a zone of a single layer, from which amœba-like processes extend upon the surface of the gelatin. These processes are constantly undergoing changes in their form and position. The young colonies deep down in the gelatin are somewhat more compact, and rounded or humpbacked; later they are covered with soft down; then they form irregular, radiating masses, and simulate the superficial colonies. But it is difficult to describe all the forms which the proteus vulgaris takes on in all the stages of its growth on gelatin plates. When the consistency of the medium is more solid, as in 10 per cent. gelatin, the liquefaction and migration of surface colonies are more or less retarded. In *gelatin-stick* cultures the growth is less characteristic—liquefaction takes place rapidly along the line of puncture, and soon the entire contents of the tube are liquefied.

Upon *nutrient agar* a rapidly spreading, moist, thin, grayish-white layer appears, and migration of the colonies also occurs. *Milk* is coagulated, with the production of acid.

Cultures in media containing albumin or gelatin have a disagreeable, putrefactive odor, and become alkaline in reaction. Growth is most luxuriant at a temperature of 24° C., but is plentiful also at 37° C. It is a facultative anaërobe and grows also in the absence of oxygen, but the proteus then loses its power of liquefying gelatin. It produces indol and phenol from peptone solutions. The proteus develops fairly well in urine, and decomposes urea into carbonate of ammonia.

Pathogenesis.—This bacillus is pathogenic for rabbits and guinea-pigs when injected in large quantities into the circulation, the abdom-

inal cavity, or subcutaneously, producing death of the animals with symptoms of poisoning. Hauser has obtained the bacillus proteus vulgaris from a case of purulent peritonitis, from purulent puerperal endometritis, and from a phlegmonous inflammation of the hand. Brunner also reports similar infections in which this organism was found associated with pus cocci, and Charrin describes a case of pleuritis during pregnancy, in which the proteus was present and a foul-smelling secretion was produced. Death in this case, which ensued without further complication, is said to have been due probably to the poisonous products of the proteus.

An interesting example of pure toxæmia resulting from the toxin of the proteus is reported by Levy: While conducting some experiments on this organism he had an opportunity of making a bacteriological examination in the case of a man who died after a short attack of cholera morbus. From the vomited material and the stools he obtained a pure culture of the proteus; but the blood, collected at the autopsy, was sterile. In the mean time seventeen other persons who had eaten at the same restaurant were taken sick in the same way. Upon examination at the restaurant it was found that the bottom of the ice-chest in which the meat was kept was covered with a slimy, brown layer, which gave off a disagreeable odor. Cultures from this gave the proteus as the principal organism present. Injections into animals of the pure cultures produced similar symptoms as occurred in the human subjects.

Levy concludes that in so-called "flesh poisoning" bacteria of this group are chiefly concerned, and that the pathogenic effects are due to toxic products evolved during their development.

Booker, from his extended researches into this subject, concludes that the proteus plays an important part in the production of the morbid symptoms which characterize cholera infantum. Proteus vulgaris was found in the alvine discharge in a large proportion of the cases examined by him, but was not found in the feces of healthy infants. "The prominent symptoms in the cases of cholera infantum in which the proteus bacteria were found were drowsiness, stupor, and great reduction in flesh, more or less collapse, frequent vomiting and purging, with watery and generally offensive stools."

Next to the bacillus coli communis the proteus vulgaris appears to be the micro-organism most frequently concerned in the etiology of pyelonephritis. In cases of cystitis and of pyelonephritis this bacillus is often found in pure cultures or associated with other bacteria. It probably gets into the bladder chiefly through catheterization. From the animal experiments of the authors above mentioned, simple injection of pure cultures of proteus into the bladder, without artificial suppression of urine, invariably produces severe cystitis. The fact that this organism grows in urine is sufficient to acconnt for the extension of the purulent process finally to the kidneys.

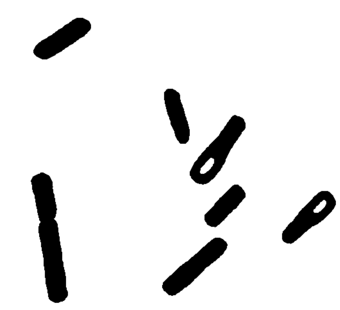
The proteus vulgaris is, however, a harmless parasite when located in the mucous membrane of the nasal cavities. Here it only decom-

poses the secretions, with the production of a putrefactive odor. On the whole, considering the very wide distribution of this organism in nature, it is remarkable how few diseases are produced by it.

The Group of Malignant Œdema Bacilli.

This group is widely distributed, being found in the superficial layers of the soil, in putrefying substances, in foul water, and by invasion from the intestine, in the blood of animals which have been suffocated. One such organism was discovered (1877) by Pasteur in animals after injections of putrefying liquids, and named by him "vibrion septique." He recognized its anaërobic nature, but did not obtain it in pure culture. Koch and Gaffky (1881) carefully studied this micro-organism, described it in detail, and gave it the name "bacillus œdematis maligni" (Fig. 115). This bacillus belongs to a group which have lateral flagellæ, produce oval spores, and grow only anaërobically.

FIG. 115



Bacillus of malignant œdema.

Morphology.—The œdema bacillus is a rod of from 0.8μ to 1μ in width, and of very varying length, from 2μ to 10μ or more, according to the conditions of its cultivation and growth. It is usually found in pairs, joined end to end, but may occur in chains or long filaments. It forms spores, and these are situated in or near the middle of the body of the rods. Exceptionally the spores are near the ends. The spores vary in length and are oval in form, being often of greater diameter than the bacilli, to which they give a more or less oval or spindle shape.

The bacilli *stain* readily by the usual aniline colors employed, but are decolorized by Gram's method.

Biology.—A strictly anaërobic, liquefying, motile bacillus. Forms spores. It grows in all the usual culture media in the absence of oxygen. Development takes place at 20° C., but more rapidly and abundantly at 37° C.

GROWTH IN GELATIN.—This bacillus may be cultivated in ordinary nutrient gelatin, but the growth is more abundant in *glucose gelatin* containing 1 or 2 per cent. of glucose. After two to three days small, almost transparent, circular colonies appear ½ to 1 mm. in diameter. Later, as liquefaction increases, the colonies become grayish and then confluent. Gas bubbles are formed and the gelatin liquefies.

GROWTH ON AGAR.—On agar plates the colonies appear as dull, whitish points, irregular in outline, and when examined under a low-power lens are seen to be composed of a dense network of interlacing threads, radiating irregularly from the centre toward the periphery.

Blood serum is rapidly liquefied, with the production of gas. Cultures of the malignant œdema bacillus give off a peculiar, diagreeable odor.

Pathogenesis.—The bacillus of malignant œdema is especially pathogenic for mice, guinea-pigs, and rabbits, although man, horses, dogs,

goats, sheep, calves, pigs, chickens, and pigeons are also susceptible. A small quantity of a pure culture injected beneath the skin of a susceptible animal gives rise to an extensive hemorrhagic œdema of the subcutaneous connective tissue, which extends over the entire surface of the abdomen and thorax, causing hyperæmia and redness of the superficial muscles. No odor is developed, and there is little, if any, production of gas. In infection with garden earth, owing to the presence of associated bacilli, the effused serum is frothy from the development of gas, and possesses a putrefactive odor. The disease, in natural infection caused by the contamination of wounds with earth or feces, runs the course above described. Simple abrasion of the skin is not sufficient to produce infection; owing to the bacillus being capable only of an anaërobic existence, the poison must penetrate deep into the tissues. Malignant œdema is confined mostly to the domestic animals, but cases have also been reported in man.

Animals which recover from malignant œdema are subsequently immune. Artificial immunity may be induced in guinea-pigs by injecting filtered cultures of the malignant œdema bacillus in harmless quantities.

In man the chief symptom is the sudden appearance of an œdematous swelling accompanied by high fever. In light cases this remains circumscribed; in severe cases it spreads widely and the case ends fatally. Autopsy shows a serous or hemorrhagic infiltration of the subcutaneous tissues and intramuscular connective tissue. In the inflamed tissue the bacilli with and without spores are found.

Bacillus Aerogenes Capsulatus.

This bacillus was found by Welch in the bloodvessels of a patient suffering with aortic aneurysm; on autopsy, made in cool weather, eight hours after death, the vessels were observed to be full of gas bubbles. Since then it has been found in a number of cases in which gas has developed from within sixty hours of death until some hours after death. These cases are, as a rule, marked by delirium, rapid pulse, high temperature, and the development of emphysema and discoloration of the diseased area, or of marked abdominal distention when the peritoneal cavity is involved.

Morphology.—Straight or slightly curved rods, with rounded or sometimes square-cut ends; somewhat thicker than the anthrax bacilli and varying in length; occasionally long threads and chains are seen. The bacilli in the animal body, and sometimes in cultures, are enclosed in a transparent capsule.

Biology.—An anaërobic, non-motile, non-liquefying bacillus. Does not form spores. Growth is rapid at 37° C., in the usual culture media in the absence of oxygen, and is accompanied by the production of gas. *Nutrient gelatin* is not liquefied by the growth of this bacillus, but it is gradually peptonized. In *agar* colonies are developed which are from 1 to 2 mm. or more in diameter, grayish-white in color, and

in the form of flattened spheres, ovals, or irregular masses, beset with hair-like projections. *Bouillon* is diffusely clouded, and a white sediment is formed. *Milk* is rapidly coagulated.

Pathogenesis.—Usually non-pathogenic in healthy animals, although Dunham found that the bacillus taken freshly from human infection is sometimes very virulent. When quantities up to 2.5 c.c. of fresh bouillon cultures are injected into the circulation of rabbits and the animals killed shortly after the injection, the bacilli develop rapidly, with an abundant formation of gas in the bloodvessels and organs, especially the liver. The following is one of the best methods of obtaining the bacilli: The material suspected to contain the bacillus alone or associated with other bacteria is injected into rabbits, which are killed, kept at 37° C., and cultures made twenty-four hours later from their bodies.

It is suggested by Welch that in some of the cases in which death has been attributed to the entrance of air into the veins the gas found at the autopsy may not have been atmospheric air, but may have been produced by this or some similar micro-organism entering the circulation and developing shortly before and after death. The bacillus has been found in the dust of hospitals.

CHAPTER XXIX.

THE ANTHRAX BACILLUS AND THE BACILLUS OF SYMPTOMATIC ANTHRAX.

Bacillus Anthracis.

ANTHRAX is an acute infectious disease which is very prevalent among animals, particularly sheep and cattle. Geographically and zoölogically it is the most widespread of all infectious disorders. It is much more common in Europe and in Asia than in America. The ravages among herds of cattle in Russia and Siberia, and among sheep in certain parts of France, Hungary, Germany, Persia, and India are not equalled by any other animal plague. Local epidemics have occasionally occurred in England, where it is known as splenic fever. In this country the disease is rare. In infected districts the greatest losses are incurred during the hot months of summer.

The disease also occurs in man as the result of infection, either through the skin, the intestines, or in rare instances through the lungs. It is found in persons whose occupations bring them into contact with animals or animal products, as stablemen, shepherds, tanners, butchers, and those who work in wool and hair. Two forms of the disease have been described—the external anthrax, or malignant pustules, and the internal anthrax, of which there are intestinal and pulmonary forms, the latter being known as "wool-sorters' disease."

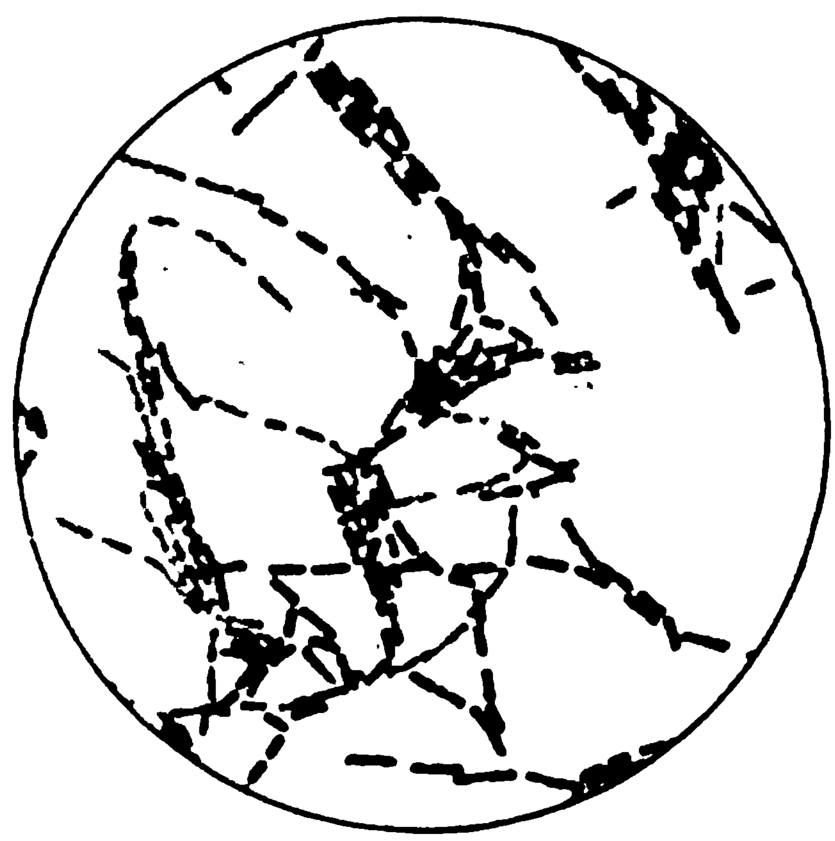
Owing to the fact that anthrax was the first infectious disease which was shown to be caused by a specific micro-organism, and to the close study which it received in consequence, this disease has probably contributed more to our general knowledge of bacteriology than any other infectious malady.

Pollender in 1849 observed that the blood of animals suffering from splenic fever always contained minute rod-shaped bacteria. Davaine in 1863 announced to the French Academy of Sciences the results of his inoculation experiments, and asserted the etiological relations of the micro-organism to the disease, with which his investigation showed it to be constantly associated. For a long time this conclusion was energetically opposed until, in 1879, Pasteur, Koch, and others established its truth by obtaining the bacillus in pure cultures, and showing that the inoculation of these cultures produced anthrax in susceptible animals as certainly as did the blood of an animal recently dead from the disease.

Morphology.—Slender, cylindrical, non-motile rods, having a breadth of 1μ to 1.25μ, and ranging from 2μ or 3μ to 20μ or 25μ in length. Some-

times short, isolated rods are seen, and, again, shorter or longer chains or threads made up of several rods joined end to end. In suitable culture media very long, flexible filaments may be observed, which are frequently united in twisted or plaited cord-like bundles. (See Fig. 116 and Fig. 13, p. 29, and Fig. 17, p. 33.) These filaments in hanging-drop cultures, before the development of spores, appear to be homogeneous or nearly so; but in stained preparations they are seen to be composed of a series of rectangular, deeply stained segments. When obtained directly from the blood of an infected animal the free ends of the rods are slightly rounded, but those coming in contact with one another are quite square. In cultures the ends are seen to be a trifle thicker than the body of the cell and somewhat concave, giving the appearance of joints of bamboo. At one time much stress was laid upon these peculiarities as distinguishing marks of the anthrax

FIG. 116



Anthrax bacillus. × 900 diameters. Agar culture.

bacillus; but it has been found that they are the effects of artificial cultivation and not necessarily characteristic of the organism under all conditions. Another peculiarity of this bacillus is that it is enclosed in a transparent envelope or capsule, which in stained preparations may be distinguished by its taking on a lighter stain than the deeply stained rods which it surrounds.

Under favorable conditions in cultures spores are developed in the bacilli. These spores are elliptical in shape and about one and a half times longer than broad. They first appear as small, refractive granules distributed at regular intervals, one in each rod. As the spore develops the mother-cell becomes less and less distinct, until it disappears altogether, the complete oval spore being set free by its dissolution. (See Fig. 117, Fig. 13, p. 29, and Fig. 17, p. 33.) Irregular sporulation sometimes takes place, and occasionally there is no spore formation, as in varieties of non-spore-bearing anthrax.

Staining.—The anthrax bacillus *stains* readily with all the aniline colors, and also by Gram's method, when not left too long in the decolorizing solution. In sections good results may be obtained by the employment of Gram's solution in combination with carmine, but when only a few bacilli are present this method is not always reliable, as some of the bacilli are generally decolorized.

Biology.—The anthrax bacillus grows easily in a variety of nutrient media at a temperature from 18° to 43° C., 37° C. being the most favorable temperature. Under 12° C. no development takes place, as a rule, though by gradually accustoming the bacillus to a lower temperature it may be induced to grow under these conditions. Under 14° C. and above 43° C. spore formation ceases. The lower limit of growth and of sporulation is of practical significance in determining the question whether development can occur in the bodies of animals dead from anthrax when buried at certain depths in the earth. Kitasato has

FIG. 117

Spores heavily stained (in specimen red). Bodies of disintegrating bacilli faintly stained (in specimen blue). $\times$ 1000 diameters

shown that at a depth of 1.5 metres the earth in July has a temperature of 15° C. at most, and that under these conditions a scanty sporulation of anthrax bacilli is possible, but that at a depth of 2 metres sporulation no longer occurs. The anthrax bacillus is aërobic—that is, its growth is considerably enhanced by the presence of oxygen—but it grows also under anaërobic conditions, as is shown by its growth at the bottom of the line of puncture in stick cultures in solid media; but under these conditions it no longer produces the peptonizing ferment which it does with free access of air. Furthermore, the presence of oxygen is absolutely necessary for the formation of spores, while carbonic acid gas retards sporulation. This explains, perhaps, why sporulation does not take place within the animal body either before or after death.

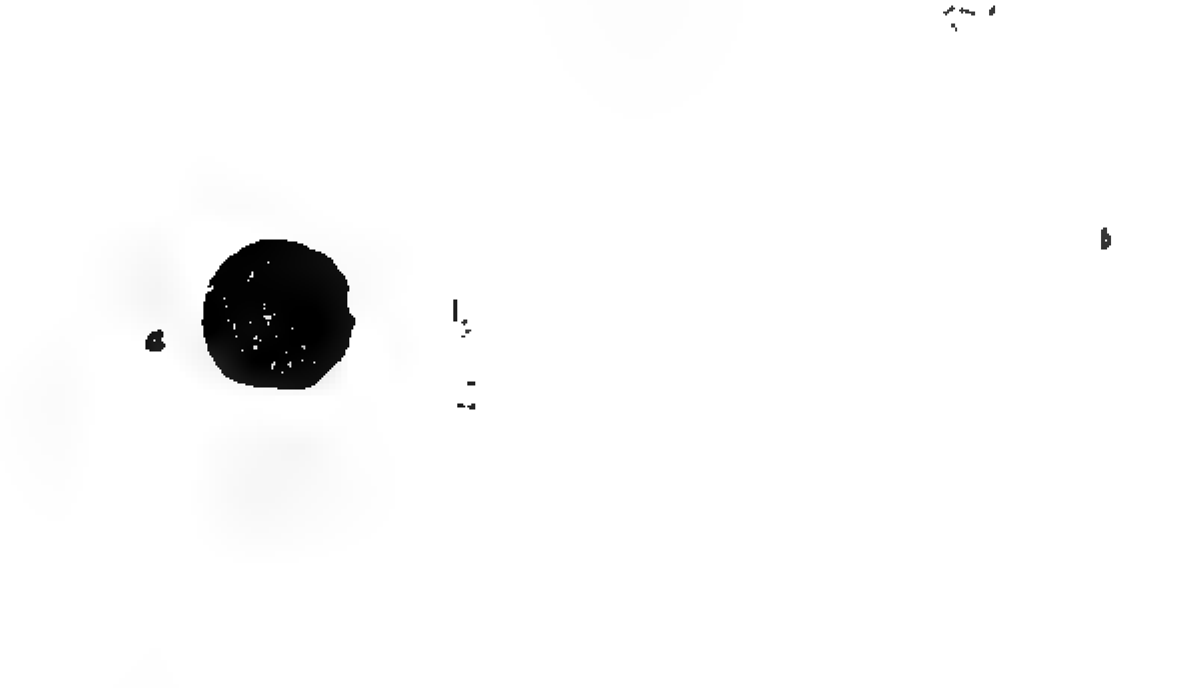
This bacillus grows best in neutral or slightly alkaline media. It may be cultivated in infusions of meat or of various vegetables, in

urine, etc., provided the reaction be not decidedly acid, which arrests development. It grows in cow-dung and in more or less contaminated earth. It is also capable of leading a saprophytic existence. The bacillus is non-motile.

Growth in Gelatin.—In *gelatin-plate cultures*, at the end of twenty-four to thirty-six hours at 24° C., small, white, opaque colonies are developed, which, under a low-power lens, are seen to be dark gray in the centre and surrounded by a greenish, irregular border, made up of wavy filaments. As the colony develops on the surface of the gelatin these wavy filaments spread out, until finally the entire colony consists of a light-gray, tangled mass, which has been likened to a Medusa head (Fig. 118).

At the same time the gelatin begins to liquefy, and the colony is soon surrounded by the liquefied medium, upon the surface of which it floats

Fig. 118



Colonies of bacillus anthracis upon gelatin plates: *a*, at the end of twenty-four hours; *b*, at the end of forty-eight hours. × 80. (F. Flügge.)

as an irregular, white pellicle. In *gelatin-stick cultures* at first development occurs along the line of puncture as a delicate white thread, from which irregular, hair-like projections soon extend perpendicularly into the culture medium, the growth being most luxuriant near the surface, but continuing also below. At the end of two or three days liquefaction of the medium commences at the surface and gradually progresses downward.

Growth on Agar.—The growth on *agar-plate cultures* in the incubator at 37° C. is similar to that on gelatin, and is still more characteristic and beautiful in appearance. A grayish-white layer is formed on the surface within twenty-four hours, which spreads rapidly and is seen to be made up of interlaced threads.

Growth in Bouillon.—The growth is characterized by the formation of flaky masses, which sink as a sediment to the bottom of the tube, leaving the supernatant liquid clear.

Spore formation, as already noted, only takes place in the presence of oxygen, and at a temperature of 15° to 43° C. There is no development of spores at a greater depth than 1.5 metres in the earth, or in the bodies of living or dead animals; but spores may be found in the fluids containing the bacilli when these come in contact with the air, as in bloody discharges from the nostrils or from the bowels of the dead animal.

There are certain non-spore bearing species of anthrax. Sporeless varieties have also been produced artificially by cultivating the typical anthrax bacillus under unfavorable conditions, among which may be mentioned the addition of antiseptics, as carbolic acid. Varieties differing in their pathogenic power may also be produced artificially. Pasteur produced an "attenuated virus" by keeping his cultures for a considerable time before replanting them upon fresh soil.

Anthrax cultures containing spores retain their vitality for years; in the absence of spores the vitality is much more rapidly lost. When grown in liquids rich in albumin the bacilli attain a considerable degree of resistance; thus dried anthrax blood has been found to retain its virulence for sixty days, while dried bouillon cultures only did so for twenty-one days. Dried anthrax spores may be preserved for many years without losing their vitality or virulence. They also resist a comparatively high temperature. Exposed in dry air they require a temperature of 140° C. maintained for three hours to destroy them; but suspended in a liquid they are destroyed in four minutes by a temperature of 100° C.

Pathogenesis.—The anthrax bacillus is pathogenic for cattle, sheep, (except the Algerian race), horses, swine, mice, guinea-pigs, and rabbits. Rats, cats, dogs, chickens, owls, pigeons, and frogs are but little susceptible to infection. Small birds—the sparrow particularly—are somewhat susceptible. Man, though subject to local infection and occasionally to internal forms of the disease, is not as susceptible as some of the lower animals.

In susceptible animals the anthrax bacillus produces a true septicæmia. Among test animals mice are the most susceptible, succumbing to very minute injections of a slightly virulent virus; next guinea-pigs, and lastly rabbits, both of these animals dying after inoculation with virulent bacilli. Infection is most promptly produced by introduction of the bacilli into the circulation or the tissues, but inoculation by contact with wounds on the skin also causes infection. It is difficult to produce infection by the ingestion even of spores; but it may readily be caused by inhalation, particularly of spores.

Subcutaneous injections of these susceptible animals results in death in from one to three days. Comparatively little local reaction occurs immediately at the point of inoculation, but beyond this there is an extensive œdema of the tissues. Very few bacilli are found in the blood in the larger vessels, but in the internal organs, and especially in the capillaries of the liver, the kidneys, and the lungs, they are present in great numbers. In some places, as in the glomeruli of the kidneys,

the capillaries will be seen to be stuffed full of bacilli, and hemorrhages, probably due to rupture of capillaries by the mechanical pressure of the bacilli which are developing within them, may occur. The pathological lesions in animals infected by anthrax are not marked except in the spleen, which, as in other forms of septicæmia, is greatly enlarged.

Occurrence in Cattle and Sheep.—Cattle and sheep are affected chiefly with the intestinal form of anthrax, infection in these animals commonly resulting from the ingestion of food containing spores. The bacillus itself, in the absence of spores, is quickly destroyed by the gastric juice. The disease usually takes a rapid course, and the mortality is high—70 to 80 per cent. The pathological lesions consist of numerous ecchymoses, enlargement of the lymphatic glands, serous, fatty, and hemorrhagic infiltration of the mediastinum and mesentery, of the mucous membranes of the pharynx and larynx, and particularly of the duodenum, great enlargement of the spleen, and parenchymatous changes in the lymphatic organs. The blood is very dark and tar-like. Bacilli are present in enormous masses.

Sheep are also subject to external anthrax, infection taking place by way of the skin; cattle are seldom infected in this way. At the point of inoculation there develops a hard, circumscribed boil—the so-called anthrax carbuncle; or there may be diffuse œdema, with great swelling of the parts. When death occurs the appearances are similar to those in intestinal anthrax, except that the duodenum is usually less affected; but in all cases metastasis occurs in various parts of the body, brought about, no doubt, by previous hemorrhages.

Occurrence in Man.—The disease does not occur spontaneously in man, but always results from infection, either through the skin, the intestines, or occasionally by inhalation through the lungs. It is usually produced by cutaneous infection through inoculation of exposed surfaces—the hands, arms, or face. Infection of the face or neck would seem to be the most dangerous, the mortality in such cases being 26 per cent.; while infection of the extremities is rarely fatal.

External anthrax in man is similar to this form of the disease in animals. There are two forms: malignant pustule or carbuncle, and, less commonly, malignant anthrax œdema.

In malignant pustule, at the site of inoculations, a small papule develops, which becomes vesicular. Inflammatory induration extends around this, and within thirty-six hours there is a dark-brownish eschar in the centre, at a little distance from which there may be a series of small vesicles. The brawny induration may be extreme. There may also be considerable œdema of the parts. In most cases there is no fever; or the temperature at first rises rapidly and the febrile phenomena are marked. Death may take place in from three to five days. In cases which recover the symptoms are slighter. In the mildest form there may be only slight swelling.

Malignant anthrax œdema occurs in the eyelids, and also in the head and neck, sometimes the hand and arm. It is characterized by the absence of the papule and vesicle forms, and by the most extensive

œdema. The œdema may become so intense that gangrene results; such cases usually prove fatal.

The bacilli are found on microscopic examination of the fluid from the pustule shortly after infection; later the typical anthrax bacilli are often replaced by involution forms. In this case resort may be had to cultures, animal inoculation, or examination of sections of the extirpated tumor. The bacilli are not present in the blood until just before death. Along with the anthrax bacilli pus cocci are often found in the pustule penetrating into the dead tissue.

Internal anthrax is much less common in man; it does, however, occur now and then. There are two forms of this: the intestinal form, or mycosis intestinalis, and the pulmonic form, or wool-sorters' disease.

Intestinal anthrax is caused by infection through the stomach and intestines, and results probably from the eating of raw flesh or unboiled milk of diseased animals. That the eating of flesh from infected animals is comparatively harmless is shown by Gerlier, who states that of 400 persons who were known to have eaten such meat not one was affected with anthrax. On the other hand, an epidemic of anthrax was produced among wild animals, according to Jansen, by feeding them on infected horse-flesh. It is evident, therefore, that there is a possibility of infection being caused in this way. The recorded cases of intestinal anthrax in man have occurred in persons who were in the habit of handling hides, hair, etc., which were contaminated with spores; in those who were conducting laboratory experiments, and rarely it has been produced by the ingestion of food, such as raw ham and milk. The symptoms produced in this disease are those of intense poisoning: chill, followed by vomiting, diarrhœa, moderate fever, and pains in the legs and back. The pathological lesions are similar to those described in animals.

Wool-sorters' disease, or pulmonic anthrax, is found in large establishments in which wool and hair are sorted and cleansed, and is caused by the inhalation of dust contaminated with anthrax spores. The attack comes on with chills, prostration, then fever. The breathing is rapid, and the patient complains of pain in the chest. There may be a cough and signs of bronchitis. The bronchial symptoms in some instances are pronounced. Death may occur in from two to seven days. The pathological changes produced are swelling of the glands of the neck, the formation of foci of necrosis in the air passages, œdema of the lungs, pleurisy, bronchitis, enlargement of the spleen, and parenchymatous degenerations.

Prophylaxis against Anthrax Infection.—Numerous investigations have been undertaken with the object of preventing infection from anthrax. The efforts of Pasteur to effect immunity in animals by preventive inoculations of "attenuated virus" of the anthrax bacillus, opened a new field of productive original research. Following in his wake many others have devised methods of immunization against anthrax infection; but the one adopted by Pasteur, Chamberland, and

Roux has alone been practically employed on a large scale. According to these authors, two anthrax cultures of different degrees of virulence attenuated by cultivation at 42° to 43° C., are used for inoculation. Vaccine No. 1 kills mice, but not guinea-pigs; vaccine No. 2 kills guinea-pigs, but not rabbits, according to Koch, Gaffky, and Loeffler. The animals to be inoculated—viz., sheep and cattle—are first given a subcutaneous injection of one to several tenths of a cubic centimetre of a four-day-old bouillon culture of Vaccine No. 1; after ten to twelve days they receive a similar dose of Vaccine No. 2. Prophylactic inoculations given in this way have been widely employed in France, Hungary, and Russia, with apparently good results.

Bacterial Cultures for Diagnosis.—The detection of the anthrax bacillus is ordinarily not difficult, as this organism presents morphological, biological, and pathogenic characteristics which distinguish it from all other bacteria. In the later stages of the disease, however, the bacilli may be absent or difficult to find, and cultivation on artificial media and experimental inoculation in animals are not always followed by positive results. Even in sections taken from the extirpated pustule it is sometimes difficult to detect the bacilli. In such cases only a probable diagnosis of anthrax can be made. It should be remembered that the bacilli are not found in the blood until shortly before death, and then only in varying quantity; thus blood examinations often give negative results, though the bacilli may be present in large numbers in the spleen, kidneys, and other organs of the body. The suspected material should be streaked over nutrient agar in Petri plates and inoculated in mice.

Differential Diagnosis.—Among other bacteria which may possibly be mistaken for anthrax bacilli are the bacillus subtilis and the bacillus of malignant œdema. The former is distinguished by its motility, by various cultural peculiarities, and by being non-pathogenic. The latter differs from the anthrax bacillus in form and motility, in being decolorized by Gram's solution, in being a strict anaërobe, and in various pathogenic properties.

The diagnosis of internal anthrax in man is by no means easy, unless the history points definitely to infection in the occupation of the individual. In cases of doubt cultures should be made and inoculations performed in animals. According to Cornil and Babes, some of these cases may possibly be caused by organisms other than the bacillus of anthrax.

Bacillus Anthracis Symptomatici (Bacillus of Symptomatic Anthrax).

Like the bacilli of anthrax and of malignant œdema, both of which it resembles in other respects also, the bacillus of symptomatic anthrax is an inhabitant of the soil. It is found as the chief cause of the disease in animals—principally cattle and sheep—known as "black leg," "quarter evil," or symptomatic anthrax (rauschbrand, German; charbon symptomatique, French), a disease which prevails in certain localities

in summer, and is characterized by a peculiar emphysematous swelling of the subcutaneous tissues and muscles, especially over the quarters.

Morphology. Bacilli having rounded ends, from 0.5μ to 0.6μ broad and from 3μ to 5μ long; mostly isolated; also occurring in pairs, joined end-to-end, but never growing out into long filaments, as the anthrax bacilli in culture and the bacilli of malignant œdema in the bodies of animals are frequently seen to do. In the hanging drop the bacilli are observed to be actively motile, and in stained preparations flagella may be demonstrated surrounding the periphery. The spores are elliptical in shape, usually thicker than the bacilli, lying near the middle of the rods, but rather toward one extremity. This gives to the bacilli containing spores a somewhat spindle shape.

Stains with the ordinary aniline dyes, but not with Gram's method, or only with difficulty and after long treatment or intense colors.

FIG. 119

Bacilli of symptomatic anthrax, showing spores. (After Zettnow.)

Biology.—Like the bacillus of malignant œdema, this is a strict anaërobe, and cannot be cultivated in an atmosphere in which oxygen is present. It grows best under hydrogen, and does not grow under carbonic acid. This bacillus develops at the room temperature in the usual culture media, in the absence of oxygen, but it grows best in those to which 1.5 to 2 per cent. of glucose or 5 per cent. of glycerin has been added.

GROWTH ON AGAR.—The colonies on agar are somewhat more compact than those of malignant œdema, but they also send out projections very often. In *agar-stick cultures*, in the incubator, growth occurs after a day or two also some distance below the surface, and is accompanied by the production of gas and a peculiar disagreeable acid odor.

Pathogenesis.—The bacillus of symptomatic anthrax is pathogenic for cattle (which are immune against malignant œdema), sheep, goats, guinea-pigs, and mice; horses, asses, and white rats, when inoculated with a culture of this bacillus, present only a limited reaction; and rabbits, swine, dogs, cats, chickens, ducks, and pigeons are, as a rule,

naturally immune to the disease. The guinea-pig is the most susceptible of test animals. When susceptible animals are inoculated subcutaneously with pure cultures of this organism, or with spores attached to a silk thread, or with bits of tissue from the affected parts of another animal dead of the disease, death ensues in from twenty-four to thirty-six hours. At the autopsy a bloody serum is found in the subcutaneous tissues, extending from the point of inoculation over the entire surface of the abdomen, and the muscles present a dark-red or black appearance, even more intense in color than in malignant œdema, and there is a considerable development of gas. The lymphatic glands are markedly hyperæmic.

The disease occurs chiefly in cattle, more rarely in sheep and goats; horses are not attacked spontaneously—*i. e.*, by accidental infection. In man infection has never been produced, though ample opportunity by infection through wounds in slaughter-houses and by ingestion of infected meat has been given. The usual mode of natural infection by symptomatic anthrax is through wounds which penetrate not only the skin, but the deep, intercellular tissues; some cases of infection by ingestion have been observed. The pathological findings present the conditions above described as occurring in experimental infection.

Distribution Outside of the Body.—Symptomatic anthrax, like anthrax and malignant œdema, is a disease of the soil, but it shows a more limited endemic distribution than the former, and is differently distributed over the earth's surface than the second of these diseases, being confined especially to places over which infected herds of cattle have been pastured. It is doubtful whether the bacilli are capable of development outside of the body like anthrax. In the form of spores, however, reproduction may take place; and by contamination with these, through deep wounds acquired by animals in infected pastures, the disease is spread.

Toxins.—Under favorable conditions extracellular toxins are formed so that the filtrate of cultures is very poisonous. Injections of the toxin into animals excite the production of antitoxins.

To recapitulate briefly, the principal points for differentiating this bacillus from the bacillus of malignant œdema, which it closely resembles, are: it is smaller; it does not develop into long threads in the tissues; it is more actively motile, and forms spores more readily in the animal body than does the bacillus of malignant œdema. It is pathogenic for cattle, while malignant œdema is not; and swine, dogs, rabbits, chickens, and pigeons, which are readily infected with malignant œdema, are not, as a rule, susceptible to symptomatic anthrax.

Preventive Inoculations.—It is well known to veterinarians that recovery from one attack of symptomatic anthrax protects an animal against a second infection. Artificial immunity to infection can also be produced in various ways: by inoculations with cultures which have been kept for a few days at a temperature of 42° to 43° C. and have thus lost their original virulence, or by inoculations of filtered cultures, or of cultures sterilized by heat. For the production of

immunity in cattle it is advised to use a dried powder of the muscles of animals which have succumbed to the disease, and which have been subjected to a suitable temperature to ensure attenuation of the virulence of the spores contained therein. Two vaccines are prepared, as in anthrax—a stronger vaccine by exposing a portion of the powder to a temperature of 85° to 90° C. for six hours, and a weaker vaccine by exposing it for the same time to a temperature of 100° to 104° C. Inoculations are made with this attenuated virus into the end of the tail—first the weaker and later the stronger. These give rise to a local reaction of moderate intensity, and the animal is subsequently immune from the effects of the most virulent material and from the disease. Fourteen days are allowed to elapse between the two inoculations. The results obtained from this method of preventive inoculation seem to have been very satisfactory. According to the statistics, including many thousand cattle treated, the mortality, which among 22,300 non-inoculated cattle was 2.20 per cent., has been reduced to 0.16 per cent. in 14,700 animals inoculated. When danger of immediate infection exists, it is advisable to inject some antitoxin with the vaccine. This lessens the reaction and gives immediate immunity.

CHAPTER XXX.

THE CHOLERA SPIRILLUM (SPIRILLUM CHOLERÆ ASIATICÆ) AND ALLIED VARIETIES.

IN 1883 Koch separated a characteristically curved organism from the dejecta and intestines of cholera patients—the so-called "comma bacillus." This he declared to be absent from the stools and intestinal contents of healthy persons, and of persons suffering from other affections. The organism was said to possess certain morphological and biological features which readily distinguished it from all previously described organisms. It was absent from the blood and viscera, and was found only in the intestines; and the greater the number, it was said, the more acute the attack. Koch also demonstrated an invasion of the mucosa and its glands by this "comma bacilli." The organisms were found in the stools on staining the mucous flakes or the fluid with methylene blue or fuchsin, and sometimes alone; by means of cultiva-

FIG. 120

Contact smear of colony of cholera spirilla from agar. × 700 diameters. (Dunham.)

FIG. 121

Cholera spirilla preparation from gelatin-plate culture of cholera. × 800 diameters.

tion on gelatin they were readily separated from the stools. During his stay in India, in Egypt, and at Toulon, Koch had examined over one hundred cases, and other investigators confirmed his statements. Numerous control observations made upon other diarrhœic dejecta and upon normal stools were negative; the comma bacillus was found in choleraic material only, or in material contaminated by cholera. Soon, however, other observers described comma-shaped organisms of non-choleraic origin. Finkler and Prior, for instance, found them in the diarrhœal stools of cholera nostras, Deneke in cheese, Lewis and Miller in saliva. All of these organisms, however, differed in many respects from Koch's comma bacillus, and it has since been proved

that none of them is affected by the specific serum of animals immunized to cholera. After a time, therefore, the exclusive association of Koch's vibrio with cholera became almost generally acknowledged until now it is regarded by bacteriologists everywhere to be the specific cause of Asiatic cholera. Certain sporadic cases of cholera-like disease, however, are undoubtedly due to other organisms.

Morphology.—Curved rods with rounded ends which do not lie in the same plane, from 0.8μ to 2μ in length and about 0.4μ in breadth. The curvature of the rods may be very slight, like that of a comma, or distinctly marked, particularly in fresh unstained preparations of full-grown individuals, presenting the appearance of a half-circle. By the inverse junction of two vibrios S-shaped forms are produced. Longer forms are rarely seen in the intestinal discharges or from cultures grown on solid media, but in fluids, especially when grown under unfavorable conditions, long, spiral filaments may develop. The spiral forms are best studied in the hanging drop, for in the dried and stained preparations the spiral character of the long filaments is often obliterated. In film preparations from the intestinal contents in typical cases it will be found that the organisms are present in enormous numbers, and often in almost pure culture (Figs. 120 and 121). In old cultures irregularly clubbed and thickened involution forms are frequent, and the presence in the organisms of small, rounded, highly refractile bodies is often noted.

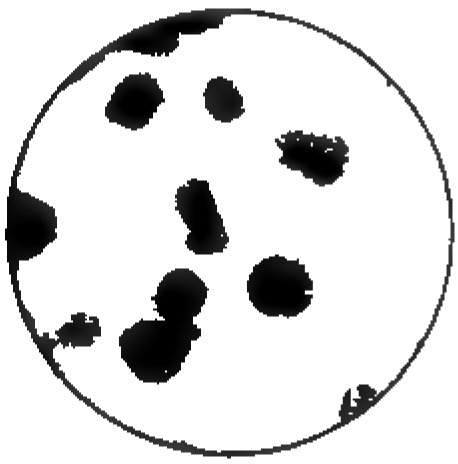
Staining.—The cholera spirillum *stains* with the aniline colors usually employed, but not as readily as many other bacteria; a dilute aqueous solution of carbol-fuchsin is recommended as the most reliable staining agent with the application of a few minutes' heat. It is decolorized by Gram's method. The organisms exhibit one long, fine, spiral flagellum attached to one end of the rods, or, exceptionally, to both ends. (Cholera-like spirilla often have 1, 2, or 3 end flagella.)

Biology.—An aërobic (facultative anaërobic), liquefying, very motile spirillum. Grows readily in the ordinary culture media, best at 37° C., but also at room temperature (22° C.); does not grow at a temperature above 42° or below 8° C. and does not form spores.

In *gelatin-plate cultures* at 22° C. the colonies are quite characteristic; at the end of twenty-four hours, small, round, yellowish-white to yellow colonies may be seen in the depths of the gelatin, which later grow toward the surface and cause liquefaction of the medium, the colonies lying at the bottom of the holes or pocket thus formed. The zone of liquefaction, which increases rapidly, at first remains clear, then becomes cloudy, mostly gray, as the result of the growth of the colonies. In many cases after a time concentric rings, increasing from day to day, appear in the zone of liquefaction. (See Figs. 122 and 123.) Examined under a low-power lens, at the end of sixteen to twenty-four hours, the colonies appear as small, light-yellow, round, coarsely granular disks, with a more or less irregular outline. In many cases at this stage an ill-defined halo is seen to surround the granular colony. As the colonies become older the granular structure increases, until a

stage is reached when the surface looks as if it were covered with little fragments of broken glass. Liquefaction continues about the colonies, their structure appears fissured and coarsely granular in texture, and occasionally a hair-like border is formed at the periphery (Fig. 123).

FIG. 122



Cholera colonies in gelatin; twenty-four to thirty-six hours' growth. × about 20 diameters.

FIG. 123

Cholera colony in gelatin. × 80 diameters. (Dunham.)

Sometimes the colonies may be retained as compact masses in the zone of liquefaction, and then they are dark yellow or brown in color, and forms occur which are absolutely unlike the typical cholera colonies.

FIG. 124

A characteristic series of cholera cultures in gelatin; one, two, three, four, and six days' growth. (Dunham.)

In *gelatin-stick cultures* the growth is at first thread-like and uncharacteristic. At the end of twenty-four to thirty-six hours a small, funnel-shaped depression appears on the surface of the gelatin, which soon spreads out in the form of an air bubble above, while below this is a

whitish, viscid mass. Later, the funnel increases in depth and diameter, and at the end of from four to six days may reach the edge of the test-tube; in from eight to fourteen days the upper two-thirds of the gelatin is completely liquefied. (See Fig. 124.) Freshly isolated cholera vibrios liquefy gelatin more rapidly than old laboratory cultures; a certain variation, under some circumstances, in the characteristic liquefaction of the gelatin even in fresh cultures, should be borne in mind in making a diagnosis. Such variations in cultural peculiarities occur also with other bacteria.

Upon the *surface of agar* the comma bacillus develops a moist, shining, grayish-yellow layer. In *agar-plate cultures*, for diagnostic purposes, the growth of separated colonies is of great importance. The nutrient agar after pouring in the plates and solidifying should be slightly dried on the surface by putting the uncovered plate face downward on the shelf of the incubator at 37° C. for thirty minutes, or at 60° C. for five minutes. The cholera colonies develop fairly characteristically, being more transparent than those of most other bacteria except the cholera-like vibrios. *Blood serum* is rapidly liquefied at the temperature of the incubator. On *potato* at incubator temperature a moist growth of a dirty-brown color occurs. *Milk* is not coagulated. In *bouillon* the growth is rapid and abundant; in the incubator at the end of ten to sixteen hours the liquid is diffusely clouded, and on the surface a wrinkled membranous layer is often formed. In general the spirillum grows in any liquid containing a small quantity of organic matter and having a slightly alkaline reaction. An acid reaction of the culture medium prevents its development, as a rule; but it has the power of gradually accommodating itself to the presence of vegetable acids. Abundant development occurs in bouillon which has been diluted with eight to ten parts of water and in simple peptone solution.

The comma bacillus belongs to the class of aërobic organisms, inasmuch as it grows readily only in the presence of oxygen, and that it develops active motility only when a certain amount of oxygen is present. It does not grow in the total absence of oxygen, but a small quantity of oxygen is all that is required for its development, as in the intestines. This need of oxygen tends to send the spirilla to the surface of fluid culture media.

Cholera-red Reaction.—When a small quantity of chemically pure sulphuric acid is added to a twenty-four-hour bouillon culture of the cholera bacillus containing peptone a reddish-violet color is produced. Brieger separated the pigment formed in this reaction—the so-called *cholera-red*—and showed that it was indol, and that the reaction was nothing more than the well-known indol reaction. Salkowski and Petri then demonstrated that the cholera bacilli produced in thin bouillon cultures, along with indol, nitrites by reducing the nitrates contained in small quantities in the culture media. They showed that it is the nitric acid, liberated by the addition of sulphuric acid to the culture, which gives rise to the indol, the red body upon which the cholera reaction depends. For a long time it was believed that this nitroso-

indol reaction was peculiar to the cholera bacillus, and great weight was placed on it as a diagnostic test. It has since been shown, however, that there are a number of other vibrios which, under similar conditions as the cholera vibrio, give the same red reaction. The reaction is, nevertheless, a constant and characteristic peculiarity of this spirillum, and is of unquestionable value. It is even more valuable as a negative than as a positive test, as the absence of the reaction enables one to say of a suspected organism that it is not the cholera spirillum. There are, however, certain precautions to be observed in its use. It has been shown that the reaction may be absent, for instance, when the culture contains either too much or too little nitrate. It is, therefore, advisable not to employ a bouillon culture the composition of which is uncertain, but a distinctly alkaline solution of peptone, containing 1 per cent. pure peptone and 0.5 per cent. of pure chloride of sodium (Dunham's solution). With such a solution constant results can be obtained.

DEVELOPMENT OUTSIDE OF THE BODY.—It has been shown by experiment that cholera spirilla multiply to some extent in sterilized river-water or well-water, and preserve their vitality in such water for several weeks or even months. Koch demonstrated the presence of this spirillum in the foul water of a tank in India which was used by the natives for drinking purposes. In his early investigations he found that rapid multiplication may occur upon the surface of moist linen.

RESISTANCE AND VITALITY.—If a culture be spread on a cover-glass and exposed to the action of the air at room temperature the bacilli will be dead at the end of two or three hours, unless the layer of culture is very thick, in which case it may take twenty-four hours or more to kill all the bacilli. This indicates that infection is not produced by means of dust or other dried objects contaminated with cholera bacilli. The transmission of these organisms through the air, therefore, can only take place for short distances, as by a spray of infectious liquids by mechanical means—as, for instance, the breaking of waves in a harbor, on water-wheels, etc., or in moist wash of cholera patients.

The cholera bacillus is also injuriously affected by the abundant growth of saprophytic bacteria. It is true that when associated with other bacteria, if present in large numbers, and if the conditions for their development are particularly favorable, the cholera bacillus may at first gain the upper hand, as in the moist linen of cholera patients, or in soil impregnated with cholera dejecta; but later, after two or three days, even in such cases, the bacilli die off and other bacteria gradually take their place. Thus, Koch found that the fluid contents of privies twenty-four hours after the introduction of comma bacilli no longer contained the living organisms; in impure river-water they were not demonstrable for more than six to seven days, as a rule. In the dejecta of cholera patients they were found usually only for a few days (one to three days), though rarely they have been observed for twenty to thirty days, and on one occasion for one hundred and twenty days. In unsterilized water they may also retain their vitality for a relatively long time; thus, in stagnant well-water they have been found for eighteen

days, and in an aquarium containing plants and fishes, the water of which was inoculated with cholera germs, they were isolated several months later from the mud at the bottom. In running river-water, however, they have not been observed for over six to eight days. For the cholera organisms the conditions favorable to growth are a warm temperature, moisture, a good supply of oxygen, and a considerable proportion of organic material. These conditions are fully met with outside the body in but very few localities.

The comma bacillus has the average resistance of spore-free bacteria, and is killed by exposure to moist heat at 60° C. in ten minutes, at 95° to 100° C. in one minute. The bacilli have been found alive kept for a few days in ice, but ice which has been preserved for several weeks does not contain living bacilli.

Chemical disinfectants readily destroy the vitality of cholera vibrios. For disinfection on a small scale, as for washing the hands when contaminated with cholera infection, a 0.1 per cent. solution of bichloride of mercury or a 2 to 3 per cent. solution of carbolic acid may be used. For disinfection on a large scale, as for the disinfection of cholera stools, strongly alkaline milk of lime is an excellent agent. The wash of cholera patients, contaminated furniture, floors, etc., may be disinfected by a solution of 5 per cent. carbolic acid and soap water.

The Spread of Cholera.—Cholera is practically always transmitted by means of water or food contaminated by the spirilla, and there is no doubt that the contamination is in most all cases through the direct soiling of the water by the feces of cholera patients. Flies which have fed or lighted on the discharges of cholera patients or on things contaminated by them have been found to carry the organisms not only on their feet, but also in their bodies for at least twenty-four hours. Food contaminated by flies is therefore a possible source of infection.

Pathogenesis.—None of the lower animals is naturally subject to cholera, nor has any contracted the disease as the result of the ingestion of food contaminated with choleraic excreta or from the inoculations of pure cultures of the spirillum, either subcutaneously or by the mouth. It has been shown that the comma bacillus is extremely sensitive to the action of acids, and is quickly destroyed by the acid secretions of the stomach of man or the lower animals, when these secretions are normally produced. Nikati and Rietsch produced a choleraic condition in a considerable percentage of dogs, where the virulent cultures were injected directly into the duodenum. Koch sought to produce infection in guinea-pigs *per vias naturales* by first neutralizing the contents of the stomach with a solution of carbonate of soda—5 c.c. of a 5 per cent. solution injected into the stomach through a pharyngeal catheter—and then after a while administered through a similar catheter 10 c.c. of a liquid into which had been put one or two drops of a bouillon culture of the comma bacillus. The animal then receives a dose of 1 c.c. of tincture of opium per 200 grams of body-weight, introduced into the abdominal cavity, for the purpose of controlling the peristaltic movements. As a result of this treatment the animals are completely nar-

cotized for about half an hour, but recover from it without showing any ill effects. On the evening of the same or the following day the animal shows an indisposition to eat and other signs of weakness, its posterior extremities become weak and apparently paralyzed, and, as a rule, death occurs within forty-eight hours with the symptoms of collapse and fall of temperature. At the autopsy the small intestine is found to be congested and filled with a watery fluid, containing the spirillum in great numbers. Koch experimented in this way on about one hundred guinea-pigs. These results, however, are somewhat weakened by the fact that experiments made with some other bacteria morphologically similar to the comma bacillus of Koch, but specifically different, occasionally produced death when introduced in the same way into the small intestines of guinea-pigs. Metchnikoff discovered that young rabbits shortly after birth could be infected by simply infecting the teats of the mother so that they received infection along with the milk.

There are several cases on record which furnish the most satisfactory evidence that the cholera spirillum is able to produce the disease in man. In 1884 a student in Koch's laboratory in Berlin, who was taking a course on cholera, became ill with a severe attack of cholera. At that time there was no cholera in Germany, and the infection could not have been produced in any other way than through the cholera cultures which were being used for the instruction of students. In 1892 Pettenkofer and Emmerich experimented on themselves by swallowing small quantities of fresh cholera cultures obtained from Hamburg. Pettenkofer was affected with a mild attack of cholerine or severe diarrhœa, from which he recovered in a few days without any serious effects; but Emmerich became very ill. On the night following the infection he was attacked by frequent evacuations of the characteristic rice-water type, cramps, tympanites, and great prostration. His voice became hoarse, and the secretion of urine was somewhat diminished, this condition lasting for several days. In both cases the cholera spirillum was obtained in pure culture from the dejecta. Another instance is reported by Metchnikoff, in Paris, of a man who became infected experimentally. In this case the algid stage of cholera was produced, with complete suppression of urine, cramps in the legs, contraction of the extremities, and collapse, the man's life being saved only with difficulty. Finally, there is the case of Dr. Oergel, of Hamburg, who accidentally, while experimenting on a guinea-pig, had some of the infected peritoneal fluid to squirt into his mouth. He was taken ill and died a few days afterward of typical cholera, though at the time of his death there was no cholera in the city. These accidents and experiments would certainly seem to prove conclusively the capability of pure cholera cultures which have retained their virulence of producing the disease.

Lesions in Man.—Cholera in man is an infective process of the epithelium of the intestine, in which the spirilla clinging to and between the epithelial cells produce a partial or entire necrosis and final destruction

of the epithelial covering, which thus renders possible the absorption of the cholera toxin formed by the growth of the spirilla. The larger the surface of the mucous membrane infected and the more luxuriant the development of bacilli and the production of toxin the more pronounced will be the poisoning, ending fatally in a toxic paralysis of the circulatory and thermic centres. On the other hand, however, there may be cases where, in spite of the large number of cholera bacilli present in the dejecta, severe symptoms of intoxication may be absent. In such cases the destruction of epithelium is not produced or is so slight that the toxic substance absorbed is not in sufficient concentration to give rise to the algid stage of the disease, or for some reason the spirilla do not produce toxin to any extent. In no stage of the disease are living cholera spirilla found in the organs of the body or in the secretions.

Distribution in the Body.—The cholera spirilla are found only in the intestines and are believed never to be present in the blood or internal organs. The lower half of the small intestine is most affected, a large part of its surface epithelium becoming shed. The flakes floating in the rice-water discharges consist mostly of masses of epithelial cells and mucus, among which are numerous spirilla. The spirilla also penetrate the follicles of Lieberkühn, and may be seen lying between the basement-membrane and the epithelial lining, which become loosened by their action. They are rarely found in the connective tissue beneath, and never penetrate deeply. In more chronic cases other micro-organisms play a greater part and deeper lesions of the intestines may occur.

Communicability.—From this fact and other known properties of the cholera spirillum, which have already been referred to, several important deductions may be made with regard to the mode of transmission of cholera infection. In the first place the bacilli evidently leave the bodies of cholera patients, chiefly in the dejections during the early part of the disease (they have usually disappeared after the fourth to the fourteenth day), and only these dejections, therefore, and objects contaminated by them, such as bed and body linen, floors, vaults, soil, well-water and river-water, etc., can be regarded as possible sources of infection. There is a special limitation even in these sources of infection, owing to the fact that this spirillum is so easily destroyed by desiccation and crowded out by saprophytic organisms. Thus, as a rule, only fresh dejections and freshly contaminated objects are liable to convey infection; after they have become completely dry there is little danger. Further, we must conclude from the distribution of the cholera bacillus in the body and from experiments upon animals that the commonest mode of infection is by way of the mouth, and chiefly by means of water used for drinking purposes, for the preparation of food, etc. In recent times cholera spirilla have been found not infrequently in water (wells, water-mains, rivers, harbors, and canals) which has become contaminated by the dejections of cholera patients.

As in like other infectious diseases, not everyone who is exposed to infection is attacked by cholera. The bacilli have been found during cholera epidemics in the dejections of healthy individuals without any pathological symptoms. Abel and Claussen for example, in 14 out of 17 persons belonging to the families of 7 cholera patients, found cholera vibrios, in some of them for a period of fourteen days. In Hamburg there were 28 such cases of healthy choleraic individuals with absolutely normal stools. It is evident, therefore, that an individual susceptibility is requisite to produce the disease. In the normal healthy stomach the hydrochloric acid of the gastric secretions may destroy the spirilla; and, finally, the normal vital resistance of the tissue cells to the action of the cholera poison may be taken into consideration. According to the greater or less power of this vital resistance of the body the same infectious matter may give rise to no disturbance whatever, a slight diarrhœa, or it may lead to serious results. Furthermore, it may be accepted as an established fact, that recovery from one attack of cholera produces personal immunity to a second attack for a considerable length of time. This does not appear to depend upon the severity of the attack; for cases are recorded of persons who were apparently not sick at all, and yet in whom an acquired immunity was produced. How long this immunity lasts is not positively known, but probably for a month or more, so that the same person is not likely to be taken ill again with cholera during an epidemic.

On the other hand, we may take it for granted that susceptibility to cholera may be acquired or increased. For instance, there is no doubt that gastric and intestinal disorders produced by overeating, etc., may act as contributing causes to the disease. Other predisposing causes are general debility from poverty, hunger, disease, etc.

Cholera Toxins.—Koch was the first to assume, as the result of his investigations, that the severe symptoms of the algid stage of cholera were due to the effects of a toxin produced by the growth of the comma bacillus in the intestines.

In 1892 Pfeiffer published an account of his elaborate researches relating to the cholera poison. He found that recent aërobic cultures of the cholera spirillum contain a specific toxic substance which is fatal to guinea-pigs in extremely small doses. There is extreme collapse, with subnormal temperature. This substance stands in close relation with the bacterial cells, and is perhaps an integral part of them. The filtrate of a recent cholera culture contains usually only moderate amounts of toxic substances. The spirilla may be killed by chloroform, thymol, or by desiccation, without apparent injury to the toxic power of this substance, but subjected to 60° C. some of the toxins are destroyed. Metchnikoff, Roux and others have shown that living, highly virulent cultures produce at times highly poisonous toxins, the 0.2 c.c. of filtrate of a three to four day culture killing 100 grams of guinea-pig. The living culture in 2 to 4 c.c. of nutrient bouillon contained in collodion sacs, when placed in the peritoneal cavity of guinea-pigs, produced symptoms of poisoning and death in a few days. Sacs containing the dead vibrios

produced little effect. There appears to be, therefore, considerable difference between the intracellular and the soluble extracellular toxins.

Cholera Immunity.—Koch found in his animal experiments that recovery from an intraperitoneal infection with small doses of living cholera vibrios produced a certain immunity against larger doses, though the animals inoculated were not very much more resistant to the cholera poison than they were originally. In 1892 Lazarus observed that the blood serum of persons who had recently recovered from an attack of cholera possessed the power of preventing the development in guinea-pigs of cholera bacilli, which in these animals are rapidly fatal when injected intraperitoneally, while the serum of healthy individuals had no such effect. This specific change in the blood is observed to take place from eight to ten days after the termination of an attack of cholera, and reaches its maximum during the fourth week of convalescence, after which it declines rapidly and disappears entirely in about two or three months. Similar antitoxic or bactericidal substances develop in the serum of guinea-pigs, rabbits, and goats, when these animals are immunized artificially against cholera by subcutaneous or intraperitoneal injections of living or dead cultures. These specific substances present in the blood of cholera-immune men and animals act only upon organisms similar to those with which they were infected; but, as Pfeiffer showed, this specific relation, which is found to exist between the antibacterial and protective substances produced during immunization and the bacteria employed to immunize the animals, is not confined to cholera. The discovery, moreover, of this specific reaction of the blood serum of immunized man and animals when brought in contact with the spirilla, has given us an apparently reliable means of distinguishing the cholera from all other vibrios, and the disease cholera from other similar affections, both of which have proved to be of great value, particularly in obscure or doubtful cases, in which heretofore the only method of differential diagnosis available—viz., by cultural tests—was often unsatisfactory.

Anticholera Inoculations.—Within the last few years Haffkine, in India, has succeeded in producing an artificial immunity against cholera infection by means of subcutaneous injections of cholera cultures. Two or three injections are necessary to give the greatest amount of protection. Animals treated by this method are refractory to intraperitoneal inoculations, but not to intestinal injections or feeding by Koch's method. In the intestines the bacteria seem to be outside the influence of the bactericidal properties of the blood, and the absorption of toxins is too great to be neutralized by the small amount of antitoxin. In over 200,000 persons whom he has inoculated the results obtained would undoubtedly seem to show a distinct protective influence in the preventive inoculations.

Agglutinins.—Five to ten days after infection (natural or experimental) agglutinins appear in the blood of man or animal. These are at least in part specific. Their presence in the blood is of diagnostic

importance. When present in great amount such agglutinins can be used for identifying doubtful spirilla.

Variations of the Cholera Spirillum.—From the great majority of all cases of epidemic cholera examined, cholera spirilla agreeing in all essential characteristics have been obtained, usually in great numbers and often in almost pure culture. In their agglutination with a specific serum they are also alike. Some cultures agglutinate with more difficulty than others, so that the same serum may agglutinate different cultures in dilutions varying from 1:1000 up to 1:10,000. Such a serum would not agglutinate cholera-like spirilla above a 1:50 dilution. Especially among isolated cases of cholera-like diseases spirilla are met with which do not agree in agglutination characteristics.

Biological Diagnosis of the Cholera Vibrio. Plan of Procedure.—*A*. Dejecta (fluid) or intestinal contents of a cholera patient or cholera suspect.

1. Use one drop (one platinum loop) for gelatin-plate cultures, making two dilutions. Do this in duplicate or triplicate. Cultivate at 22° C.
2. Inoculate a couple of bouillon tubes and a couple of Dunham's 1 per cent. peptone solution with one drop each, and place them in the incubator (37° to 38° C.) for six to eight hours.
3. Examine a drop of the dejecta in the hanging drop.
4. Examine a drop of the dejecta in stained cover-glass preparation.[1]
5. Make gelatin plates from one drop taken from the surface of each of the bouillon and peptone solution tubes and cultivate at 22° C.
6. As soon as the plates (see 1 and 5) are sufficiently developed (thirty-six to forty-eight hours) fish the suspected cholera colonies and use the material for the following procedures:
7. Inoculate six or eight peptone tubes (1 per cent. peptone and 0.5 per cent. NaCl in distilled water) and place them at once in the incubator. Note the time.
8. Examine hanging drop for form, size, and motility (and arrangement).
9. Make stained cover-glass preparations and examine.
10. Then try indol reaction with the same tubes.
11. While these tubes are incubating use material from the suspected colonies on the plates (1 and 5) for hanging-drop cultures.
12. Meanwhile make stained cover-glass preparations from other colonies of suspected cholera on the plates (1 and 5).
13. Make gelatin-tube cultures from colonies on plates (1 and 5).
14. Make gelatin-tube cultures daily for five or six days, to study shape of growth along the line of puncture to preserve the culture.

[1] These direct microscopic examinations of the intestinal contents are, as a rule, very unsatisfactory, at least in those in which the symptoms are not marked. In a few the spirals will make up from 50 to 100 per cent. of the bacteria present. In most of the cases during the last epidemic in New York Dunham found abundance of columnar epithelium from the intestinal mucous membrane, numerous straight, thick bacilli, and only a few curved bacilli or segments of spirals—too few to identify. Plate cultures from these showed from 20 to 80 per cent. of all the colonies developing to be cholera spirilla.

B. Suspected water.

Add to 500 c.c. or 1 litre of the water to be examined in a flask half-full enough peptone-salt solution (20 per cent. peptone and 10 per cent. NaCl) to make a 1 per cent. solution of peptone. Then proceed as in *A*.

Specific Serum Reactions.—All authors now agree that the differentiation of the cholera vibrio from other similar vibrios cannot always be made by the cultural method, nor is the usual inoculation of animals sufficient. For this purpose serum is employed either by making intraperitoneal injections of a surely fatal dose of the suspected spirillum along with the serum of animals immunized to undoubted cholera cultures, or to note whether specific protection is afforded, or the Gruber-Widal test is carried out in such a way as to determine if specific agglutination of the spirilla occurs.

Spirilla More or Less Allied to the Cholera Spirillum.

The examinations of the stools of persons suffering from cholera have revealed, in a small percentage of cases, spirilla resembling either very closely or having a fair degree of similarity to the true cholera organisms. Further, in a small percentage of cases having choleraic symptoms no true cholera vibrios have been found, but instead other spirilla resembling them more or less closely.

These may differ in having two or more end flagella, in size, in production of nitrites, etc., or they may be identical in the tests commonly employed. They all differ in the specific agglutination and bacteriolytic tests from the cholera spirilla and among themselves.

In a recent epidemic in Egypt, Gottschlich obtained from sixteen cases spirilla differing from the true spirilla, and found every one distinct in some characteristic from all others. Some were pathogenic for pigeons, through inoculation of a small quantity into the breast muscle; others were atypical in their development in nutrient gelatin. None of these micro-organisms injected into animals induced production of agglutinins for the true cholera spirilla.

Kolle and Gottschlich consider these various spirilla found by them in Egypt as well as others found by different investigators in India, Germany, and elsewhere to be saprophytes. It is more probable, in the writer's opinion, that some of them must be considered as bearing a part in exciting a cholera-like disease, but that they are not very pathogenic and require very favorable conditions before they can exert their action.

Some special varieties of spirilla resembling those of cholera have received especial attention on account of having been obtained before it was known that so many cholera-like vibrios existed. The vibrio Berolinensis, cultivated by Neisser from Berlin sewage-water; the vibrio Danubicus, cultivated by Hausser from canal-water, and the vibrio of Massowah, cultivated by Pasquale from a case during an epidemic of cholera, all are negative to the specific serum reactions,

and differ in the number of terminal flagella or in other characteristics. Cunningham found a number of such spirilla in cases of apparently true cholera in India. Some of these may have been true cholera spirilla and others may have had some relationship to the disease in the person from which they were derived.

Spirillum of Finkler and Prior.

Because of their prominence in literature and their frequent use in teaching, the spirillum of Finkler and Prior, that of Metchnikoff, and that of Deneke are of considerable interest.

Finkler and Prior, in 1884, obtained from the feces of patients with cholera nostras, after allowing the dejecta to stand for some days, a spirillum which is of interest mainly because it simulates the comma bacillus of Koch, but differs from it in several cultural peculiarities.

FIG. 125

Spirillum of Finkler and Prior. × 1100 diameters.

Morphology.—Somewhat longer and thicker than the spirillum of Asiatic cholera and not so uniform in diameter, the central portion being usually wider than the pointed ends.

Biology.—An aërobic and facultative anaërobic, liquefying spirillum. Does not form spores. Upon *gelatin plates* small, white, punctiform colonies are developed at the end of twenty-four hours. These are round, but less coarsely granular, darker in color, and with a more sharply defined border than the comma bacillus. Liquefaction of the gelatin around these colonies progresses rapidly, and at the end of forty-eight hours is usually complete in plates where they are numerous. In *gelatin-stick cultures* liquefaction progresses much more rapidly than in similar cultures of the cholera spirillum, and a stocking-shaped pouch of liquefied gelatin, already seen after forty-eight hours, is filled with a cloudy liquid. The liquefaction increases, and in twenty-four hours more reaches the sides of the tube in the upper part of the medium; by the end of the week the gelatin is usually completely liquefied. Upon the surface of the liquefied medium a whitish film is seen.

Upon *agar* there is a somewhat more luxuriant growth than is seen with the cholera vibrio. Upon *potato* this spirillum grows at room temperature and produces a slimy, grayish-yellow, glistening layer which soon extends over the entire surface. The cholera spirillum does not grow at room temperature, and in the incubator produces a thin, brownish layer. The absence of agglutination with a suitable dilution of the serum of an animal immunized to the cholera spirillum is a valuable differential sign.

In 1884 Miller observed a curved bacillus in a hollow tooth, which from its behavior in microscopic preparations, in cultures, and animal experiments, is probably identical with the Finkler and Prior spirillum. Very similar spirilla have been found by others.

Spirillum of Metchnikoff.

Discovered in 1888, in Odessa, by Gamaleïa in the intestinal contents of fowls dying of an infectious disease, which prevails in certain parts of Russia during the summer months, and which presents symptoms resembling fowl cholera. Gamaleïa's experiments show that this organism is the cause of the disease mentioned. It has since been found by Pfuhl and Pfeiffer in the water of the Spree at Berlin, and in the Lahn by Kutchler.

Morphology.—Morphologically this spirillum is almost identical with the cholera spirillum. In the blood of inoculated pigeons the diameter is sometimes twice as great as that of the cholera spirillum, and almost coccus-like forms are often found. A single, long, undulating flagellum is attached to one end of the spiral filaments or curved rods.

Stains with the usual aniline colors, but not by Gram's method.

Cultural Characters.—Upon *gelatin plates* the vibrio Metchnikoff grows considerably faster than the cholera vibrio; small, white, punctiform colonies are developed at the end of twelve hours; these rapidly increase in size and cause liquefaction of the gelatin within twenty-four to thirty hours. At the end of three days large, saucer-like areas of liquefaction may be seen, the contents of which are turbid, as a rule. In *gelatin-stick cultures* the growth is almost twice as rapid as the cholera bacillus. In *bouillon* at 37° C. development is very rapid, and the liquid becomes clouded and opaque, and a thin, wrinkled film forms upon the surface. On the addition of pure sulphuric acid to twenty-four-hour peptone cultures a distinct nitrosoindol reaction is produced. *Milk* is coagulated and acquires a strongly acid reaction. The spirillum is not agglutinated by the specific cholera agglutinin.

Pathogenesis. — The vibrio Metchnikoff is pathogenic for fowls, pigeons, and guinea-pigs. A small quantity of a virulent culture fed to chickens and pigeons causes their death with the local and general symptoms of fowl cholera. At the autopsy the most constant appearance is hyperæmia of the entire alimentary canal. A grayish-yellow liquid, more or less mixed with blood, is found in considerable quantity

in the small intestine. In the watery fluid large numbers of spirilla are found. A few drops of a pure culture inoculated subcutaneously in pigeons produce septicæmia and cause their death in twelve to twenty-four hours.

In contradistinction to the pathogenic virulence of these spirilla for pigeons and guinea-pigs, the cholera spirillum is much less pathogenic. Pigeons are not killed by the intramuscular inoculation of pure fresh cultures of the vibrio choleræ. The pathogenic action of the vibrio Metchnikoff upon pigeons and guinea-pigs, producing in these animals general septicæmia and death, is, therefore, a characteristic point of difference between this and the spirillum of Asiatic cholera.

Within recent years numerous other spirilla, the so-called "water vibrios," have been found while looking for the cholera spirillum.

CHAPTER XXXI.

GLANDERS BACILLUS (BACILLUS MALLEI).

THIS bacillus was discovered and proved to be the cause of glanders, by isolation in pure culture and communication to animals by inoculation, by several bacteriologists almost at the same time (1882). The bacilli were first obtained in impure cultures by Bouchard, Capitan, and Charrin, and first accurately studied in pure culture by Loeffler and Schütz. They are present in the recent nodules in animals affected with glanders, and in the discharge from the nostrils, pus from the specific ulcers, etc., and occasionally in the blood.

Morphology.—Small bacilli with rounded or pointed ends, from nutrient agar cultures, 0.25μ to 0.5μ broad and from 1.5μ to 5μ long; usually single, but sometimes united in pairs, or growing out to long filaments, especially in potato cultures. The bacilli frequently break up into short, almost coccus-like elements (Fig. 126).

Staining.—The bacillus mallei *stains* with difficulty with the aniline colors, best when the aqueous solutions of these dyes are made feebly alkaline; it is decolorized by Gram's method. This bacillus presents the peculiarity of losing very quickly in decolorizing solutions the color imparted to it by the aniline-staining solutions. For this reason it is difficult to stain in sections. Loeffler recommends his alkaline methylene-blue solution for staining sections, and for decolorizing a mixture containing 10 c.c. of distilled water, 2 drops of strong sulphuric acid, and 1 drop of a 5 per cent. solution of oxalic acid; thin sections to be left in this acid solution for five seconds.

Biology.—An aërobic, non-motile bacillus, whose molecular movements are so active that they have often been taken for motility. It grows on various culture media at 37° C. Development takes place slowly at 22° C. and ceases at 43° C. The bacillus does not form spores. Exposure for ten minutes to a temperature of 55° C., or for five minutes to a 3 to 5 per cent. solution of carbolic acid, or for two minutes to a 1:5000 solution of mercuric chloride, destroys its vitality. As a rule, the bacilli do not grow after having been preserved in a desiccated condition for a week or two; in distilled water they are also quickly destroyed. It is doubtful whether the glanders bacillus finds conditions in nature favorable to a saprophytic existence.

Cultivation. (For methods of separation see page 411.)—It grows well in the incubating oven on *glycerin agar*. Upon this medium at the end of twenty-four to forty-eight hours, whitish, transparent colonies are developed, which in six or seven days may attain a diameter of 7 or 8 mm. On *blood serum* a moist, opaque, slimy layer develops,

which is of a yellowish-brown tinge. The growth on cooked *potato* is especially characteristic. At the end of twenty-four to thirty-six hours at 37° C. a moist, yellow, transparent layer develops; this later becomes deeper in color, and finally takes on a reddish-brown color, while the potato about it acquires a greenish-yellow tint. In *bouillon* the bacillus causes diffuse clouding, ultimately with the formation of a more or less ropy, tenacious sediment. It grows on media possessing a slightly acid reaction, and both with and without oxygen. *Milk* is coagulated with the production of acid.

Pathogenesis.—The bacillus of glanders is pathogenic for a number of animals. Among those which are most susceptible are horses, asses, guinea-pigs, cats, dogs, ferrets, moles, and field mice; sheep, goats, swine, rabbits, white mice, and house mice are much less susceptible; cattle are immune. Man is susceptible, and infection not infrequently terminates fatally.

FIG. 126

Glanders bacilli. Agar culture. × 1100 diameters.

When pure cultures of the bacillus mallei are injected into horses or other susceptible animals true glanders is produced. The disease is characterized in the horse by the formation of ulcers upon the nasal mucous membrane, which have irregular, thickened margins, and secrete a thin, virulent mucus; the submaxillary lymphatic glands become enlarged and form a tumor which is often lobulated; other lymphatic glands become inflamed, and some of them suppurate and open externally, leaving deep, open ulcers; the lungs are also involved, and the breathing becomes rapid and irregular. In farcy, which is a more chronic form of the disease, circumscribed swellings, varying in size from a pea to a hazel-nut, appear on different parts of the body, especially where the skin is thinnest; these suppurate and leave angry-looking ulcers with ragged edges, from which there is an abundant purulent discharge. The bacillus of glanders can easily be obtained in pure cultures from the interior of suppurating nodules and glands

which have not yet opened to the surface, and the same material will give successful results when inoculated into susceptible animals. The discharge from the nostrils or from an open ulcer may contain comparatively few bacilli, and these being associated with other bacteria which grow more readily on the culture media than the bacillus mallei, make it difficult to obtain pure cultures from such material by the plate method. In that case, however, guinea-pig inoculations are useful.

Of test animals guinea-pigs and field mice are the most susceptible. In guinea-pigs subcutaneous injections are followed in four or five days by swelling at the point of inoculation, and a tumor with caseous contents soon develops; then ulceration of the skin takes place, and a chronic purulent ulcer is formed. The essential lesion is the granulomatous tumor, characterized by the presence of numerous lymphoid and epithelioid cells, among and in which are seen the glanders bacilli. The lymphatic glands become inflamed and general symptoms of infection are developed in from two to four weeks; the glands suppurate and in males the testicles are involved; finally purulent inflammation of the joints occur, and death ensues from exhaustion. The formation of the specific ulcers upon the nasal mucous membrane, which characterizes the disease in the horse, is rarely seen when guinea-pigs are inoculated. In these the process is often prolonged, or remains localized on the skin. They succumb more rapidly to intraperitoneal injection, usually in from eight to ten days, and in males the testicles are invariably affected.

Mode of Spread.—Glanders occur as a natural infection only in horses and asses; the disease is occasionally communicated to man by contact with affected animals, usually by inoculation on an abraded surface of the skin. The contagion may also be received on the mucous membrane. Infection has sometimes been produced in bacteriological laboratories. In man, an acute and chronic form of glanders may be recognized, and an acute and a chronic form of farcy. The disease is fatal in about 60 per cent. of the cases. It is transmissible also from man to man. Washerwomen have been infected from the clothes of a patient. The infective material exists in the secretions of the nose, in the pus of glanders nodules, and frequently in the blood; it may occasionally be found in the secretions of glands not yet affected, as in the urine, milk, and saliva, and also in the fœtus of diseased animals (Bonome). From recent observations it appears that glanders is by no means an uncommon disease among horses, particularly in southern countries, sometimes taking a mild course and remaining latent for a considerable time. Horses apparently healthy, therefore, may possibly spread the disease.

Attenuation of virulence occurs in cultures which have been kept for some time, and inoculations with such cultures may give a negative result, or, when considerable quantities are injected, may produce a fatal result at a later date than is usual when small amounts of a recent culture are injected.

Immunity.—Attempts have been made to produce artificial immunity against glanders, but so far with unsatisfactory results. According to Strauss, by intravenous inoculations of small quantities of living bacilli, dogs may be protected against an injection of quantities which usually kill them. Fenger has found that animals inoculated with glanders bacilli react less powerfully to fresh injections; and that rabbits which have recovered from an injection of glanders are subsequently immune, the immunity lasting for from three to six weeks. Ladowski has obtained positive results also in rabbits and cats by intravenous injections of sterilized cultures. Other observers have reported not only the production of immunity, but also cures, by the use of *mallein*. This is prepared in the same way as tuberculin. It consists of the glycerinated bouillon in which the glanders bacilli have grown and which contains the products of their growth and activity. Concentrated mallein is produced by evaporating a six-weeks-old culture of the glanders bacillus in 5 per cent. glycerin nutrient veal bouillon to 10 per cent. of its original bulk. Some evaporate the culture fluid only to 20 per cent. The dose is about 0.5 c.c. of the former, or 2 c.c. of the second preparation.

Use of Guinea-pigs and Cultures in Diagnosis.—It is often difficult to demonstrate microscopically the presence of the bacillus of glanders in the nodules which have undergone purulent degeneration, in the secretions from the nostrils, or in the pus from the specific ulcers and suppurating glands. It is then necessary to make immediate cultures and also animal tests of these discharges by inoculating susceptible animals, as guinea-pigs and mice, and then from these to obtain a pure culture; but this requires time, and in clinical work it is of great importance for the diagnosis to be established as quickly as possible. With this view Strauss has prepared a method which is prompt and which has given very satisfactory results. This consists in introducing into the peritoneal cavity of a male guinea-pig some material or a culture from the suspected products. If it be a case of glanders, the diagnosis may be made within two or three days from the tumefaction of the testicles, which become red and swollen, and show evidences of pus formation. One objection to this method, however, is that occasionally from the injection of impure material, as in the nasal secretion, the animal may die of septicæmia. This is particularly frequent when field mice are used for the tests; but if pure matter can be obtained, as from the lymphatic glands of the horse, this method is entirely satisfactory.

Diagnostic Use of Mallein.—The diagnosis of glanders in horses, in which the usual symptoms of the disease have not yet manifested themselves, or in which it is suspected, may often be made by the use of mallein. Following an injection of mallein in a glanderous horse (best made about midnight) there will be a local reaction, and a general reaction with a rise of temperature. The temperature usually begins to rise three or four hours after the injection, and reaches its maximum between the tenth and twelfth

hour. Sometimes, however, the highest point is not reached until fifteen or eighteen hours after the injection. This elevation of temperature is from 1.5° to 2° C. (2° to 3.5° F.), above the normal mean temperature. In a healthy animal the rise of temperature, as a rule, amounts to only a few tenths of a degree, but it may reach 1° C. The rise of temperature, however, should be considered always in connection with the general and local reactions. In a glanderous animal, after an injection of mallein, the general condition is more or less profoundly modified. The animal has a dejected appearance; the countenance is pinched and anxious, the hair is rough, the flank is retracted, the respirations are rapid, there are often rigors, and the appetite is gone. In healthy animals the general symptoms do not occur. The local reaction around the point of injection in a glanderous animal is usually very marked. A few hours after the injection there appears a large, warm, tense, and very painful swelling, and running from this will be seen hot, sensitive lines of sinuous lymphatics, directed toward the neighboring lymphatic nodes. This œdema increases for twenty-four to thirty-six hours and persists for several days, not disappearing entirely for eight or ten days. In healthy animals, at the point of injection, mallein produces only a small œdematous tumor, and the œdema, instead of increasing, diminishes rapidly and disappears within twenty-four hours. The value of this test has been demonstrated by numerous experiments. There are some exceptions to the rule as described above, but they are infrequent, and mallein has been used with considerable success as a diagnostic aid in detecting the existence or absence of glanders in doubtful or obscure cases.

CHAPTER XXXII.

THE BACILLUS OF BUBONIC PLAGUE—THE BACILLUS ICTEROIDES —THE MICROCOCCUS MELITENSIS.

Bacillus of Bubonic Plague (Bacillus Bacterium Pestis Bubonicæ).

HISTORICALLY we can trace the bubonic plague back to the third century. In Justinian's reign a great epidemic spread over the Roman empire and before it terminated destroyed in many portions of the country nearly 50 per cent. of the people. Among the most fatal forms of infection is that of the lungs. Pneumonic cases are not alone very serious, but they readily spread infection. The bacillus exciting the disease was discovered simultaneously by Kitasato and Yersin (1894) during an epidemic of the bubonic plague in China. It is found in large numbers in the seropurulent fluid from the recent buboes char-

FIG. 127

Bacilli from agar culture. × 1100 diam.

FIG. 128

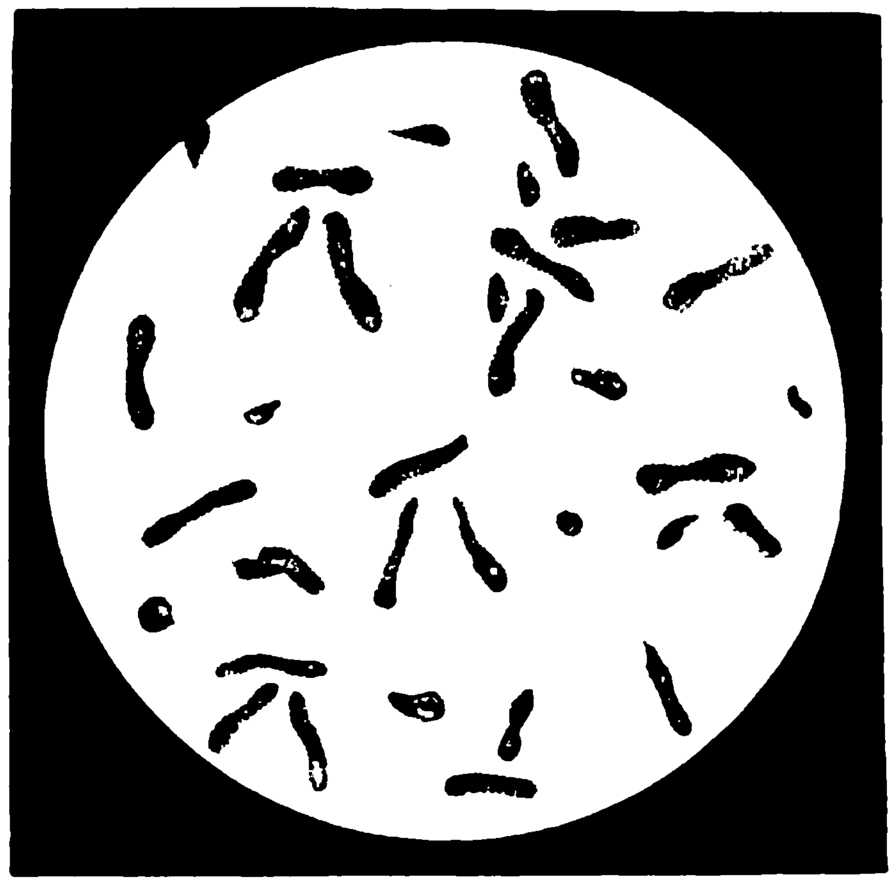
Bacilli from bouillon culture. × 1100 diam.

acteristic of this disease and in the lymphatic glands; more rarely in the internal organs and in the blood, in which it occurs in acute hemorrhagic cases and shortly before death. It also occurs in malignant cases in the feces of men and animals. The bacillus is closely allied to the hemorrhagic septicæmia group.

Morphology.—The bacilli in smears from acute abscesses or infected tissues are, as a rule, short, thick rods with rounded ends. The central portion of the bacillus is slightly convex. When lightly stained the two ends are more colored than the middle portion. The bacilli are mostly single or in pairs. Bacilli in short chains occur at times. The

length of the bacilli varies, but on the average is about 1.6μ (1.5μ to 1.7μ), breadth 0.5μ to 0.7μ. Besides the usual oval form the plague bacillus has many exceptional variations which are characteristic of it. In smears especially from old buboes one looks for long bacilli with clubbed

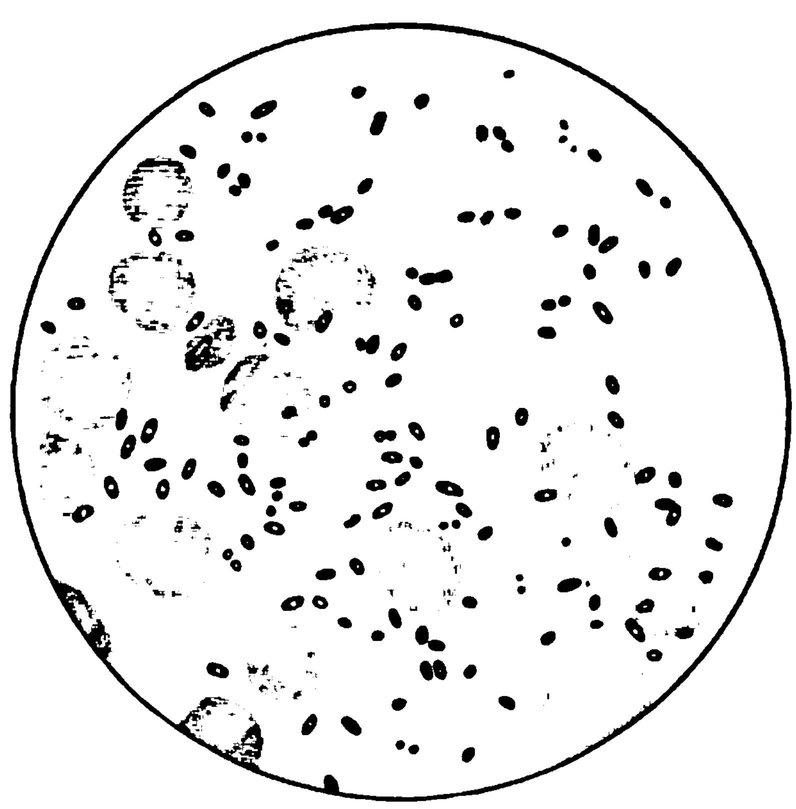
FIG. 129



Involution forms on salt agar. (Kolle and Wassermann.)

ends (similar to involution forms, Fig. 129), yeast-like forms, and bladder shapes. Some of these stain with difficulty. When obtained from cultures the bacilli present not only the forms already mentioned, but also long chains.

FIG. 130



Bacilli in acutely inflamed gland.

Staining.—They *stain* readily with the ordinary aniline dyes, and especially well with methylene blue, the ends being usually more deeply colored than the central portion; does not stain by Gram's method.

Biology.—An aërobic, non-motile bacillus. Grows best at 30° to 35° C. Does not form spores. Grows on the usual culture media, which should have a slightly alkaline reaction. Does not liquefy gelatin. Grows well on *blood-serum* media. It grows rapidly on *glycerin agar*, forming a grayish-white surface growth. The bacilli appear, as a rule, as short, plump, oval bacilli, but a few present elongated thread forms which are very characteristic. In *bouillon* a very characteristic appearance is produced, the culture medium remaining clear while a granular or grumous deposit forms on the walls and on the bottom of the tube. In bouillon and most fluid media the growth is in the form of short or medium chains of very short, oval bacilli, which look almost like streptococci.

Pathogenesis.—This bacillus is pathogenic for rats, mice, guinea-pigs, monkeys, rabbits, flies, and other insects, which usually die within two or three days after inoculation. Then at the point of inoculation is found a somewhat hemorrhagic infiltration and œdema, with enlargements of the neighboring lymph glands, hemorrhages into the peritoneal cavity, and parenchymatous congestion of the organs. The spleen sometimes shows minute nodules resembling miliary tubercles. Microscopically the bacilli are found in all the organs and in the blood. The disease is rapidly communicated from one animal to another, and thus its extension is facilitated. During epidemics, rats, mice, and flies, in large numbers, become infected and die, and the disease is frequently transmitted through them to man. The organism is found at times in the feces of sick animals, in the dust of infected houses, and in the soil.

The virulence of the bacilli in cultures and in nature seems to vary considerably, and rapidly diminishes when grown on artificial media. The growth in cultures becomes more abundant after frequent transplantation. The virulence of the organism is increased by successive inoculation in certain animal species, and then its pathogenic properties for other species are less marked.

Yersin, Calmette, and Borrel have succeeded in immunizing animals against the bacillus of bubonic plague by inoculation, by the intravenous or intraperitoneal injection of dead cultures, or by repeated subcutaneous inoculation. They also succeeded in immunizing rabbits and horses, so that the serum afforded protection to small animals, after subcutaneous injection of virulent cultures, and even cured those which had been inoculated, if administered within twelve hours after injection. The serum has considerable antitoxic as well as bactericidal properties. More recently this serum has been applied to the treatment of bubonic plague in man, with promising results. Experience has shown that the treatment is more efficacious the earlier the stage of the disease. When treatment is begun in the first day of the attack, fever and all alarming symptoms frequently disappear with astonishing rapidity. In cases treated at a later stage larger doses of the serum are required, and even in the favorable cases suppuration of the buboes is not always prevented. In some of the early cases and in many of

the rather late ones the serum fails. When the disease is far advanced the serum is powerless. For immunizing purposes the serum should be valuable, and a single injection would probably give protection for several weeks.

Haffkine, in India, has recently applied his method of preventive inoculation to the bubonic plague, as he previously did with cholera, and apparently with equally good results. This method consists in an inoculation of dead cultures, and is essentially a protective rather than a curative treatment. It gives after six to ten days a considerable immunity, lasting a month or more. By means of these two methods of inoculation, along with strict quarantine regulations, it is to be hoped that this disease which under the name of Black Death once decimated the populations of the earth, and which in the East still causes great mortality at times, may finally be greatly restricted.

Duration of Life Outside of the Body.—In cultures protected from the air and light the plague bacilli may live one year or more. In the bod es of dead rats they may live for two months. In sputum from pneumonic cases the bacilli lived ten days. Upon sugar sacks, food, etc., they may live six to fifteen days.

Resistance to Deleterious Influences.—The bacilli resemble the colon bacilli in their reaction to heat and disinfectants. Boiling for one to two minutes kills them. Carbolic acid, 5 per cent. solution, kills culture in one minute, in 2½ per cent. in two minutes, etc.

Bacteriological Diagnosis.—When the lymph glands are acutely inflamed but not yet suppurated cut down on one and make cultures on nutrient agar slanted in tubes. If pus has formed withdraw a little by means of the hypodermic needle. There should also be made smears from the suspected bubo, or in case of pneumonia from the sputum. If the patient is dead, cultures from the spleen and heart's blood are also taken when possible. Suspected animals, such as rats and mice, when freshly killed, are examined as in man; when decomposed, rats and guinea-pigs should be inoculated.

Bacillus Icteroides.

In 1897 Sanarelli announced the discovery of a micro-organism which he claimed to be the specific cause of yellow fever. This he called the "bacillus icteroides." It is found in the circulating blood and in the tissues of most yellow fever patients.

Morphology.—It resembles the colon bacilli in many characteristics. The work of Reed and his associates having thoroughly overthrown the claims of Sanarelli, its description is omitted with the exception of a few notes.

It *stains* readily with the ordinary aniline dyes, but not by Gram's method.

Biology.—A motile, facultative, anaërobic, non-liquefying bacillus. Does not form spores as far as known. Grows readily in all the ordinary culture media at the room temperature, but best at 37° C. in the incu-

bator. Cultures on *agar* at 20° C. are characteristic, according to Sanarelli. Grown at room temperature they appear like drops of milk, opaque, projecting, and with pearly reflections.

The bacillus icteroides ferments glucose and saccharose, but does not coagulate milk.

Pathogenesis.—It is pathogenic for the greater number of the domestic animals; but birds are completely refractory.

CHAPTER XXXIII.

REPRESENTATIVE PATHOGENIC MICRO-ORGANISMS BELONGING TO THE HIGHER BACTERIA.

THE members of the higher bacteria which are pathogenic to man have as yet been incompletely studied and classified. The following divisions serve as an attempt at differentiation:

1. *Actinomyces* is characterized by the radiating wreath-like forms which it alone produces in the living body.

2. *Streptothrix*, by its abundant true branching, wavy growth, later fragmentation, and formation of conidiæ, which serve as organs of propagation, and in this sense may be considered as spores.

3. *Cladothrix*, by its false branching, rapid fragmentation, and then bacillary characteristics in old cultures.

4. *Leptothrix*, by its lack of observed branching, non-wavy growth, but, on the contrary, stiff, almost straight threads, in which division processes are seldom or never observed.

These higher bacteria may rightly be considered, according to their development, as a transition group between the simple bacteria and the more highly developed fungi.

The streptothrix group of micro-organisms while having many affinities with the bacteria, yet differs from them in many important respects which link them with the fungi. They develop from spore-like bodies into cylindrical dichotomously branching threads, which grow into colonies, the appearance of which suggests a mass of radiating filaments. Under favorable conditions certain of the threads become fruit hyphæ, and these break up into chains of round, spore-like bodies, which do not, however, have the same staining reactions nor resisting powers as true spores. The tubercle grass and diphtheria bacilli are by some believed to properly belong in the streptothrix group, on account of the true branching forms developed by them under certain conditions. The actinomyces fungus is by some classed in the streptothrix group.

The Micro-organism of Actinomycosis.

This parasite was first discovered by Bollinger in the ox and given the name of actinomyces, or ray fungus, by the botanist Harz. The two most important publications on the subject of the biology of this micro-organism are those of Bostroem and of Wolf and Israel, published in 1890 and 1891, respectively.

The characteristics of the micro-organism described by these workers differed greatly and have led to confusion. Bostroem's organism grew best aërobically and developed well at room temperature. He noted the intimate relation of the organism with fragments of grain, and this led to the finding of similar micro-organisms in the outer world on grains, grasses, etc.

There is no doubt that some suppurative processes have been due to organisms of these characteristics, but they do not seem to excite true actinomycosis.

Wolf and Israel described a micro-organism from two human cases, which differ from that described by Bostroem, but agrees with the micro-organisms obtained by most of the more recent investigators. It grew best under anaërobic conditions and did not grow at room temperature. Its growth was much less luxuriant than Bostroem's micro-organism. On the surface of anaërobic agar slant cultures on the third, fourth, and fifth day numerous minute isolated dew-drop-like colonies appeared, the largest pinhead in size. These gradually became larger and formed ball-like, irregularly rounded elevated nodules varying in size up to that of a millet-seed, exceptionally attaining the size of a lentil or larger. As a rule the colonies did not become confluent, and an apparently homogeneous layer of growth was seen to be made up of separate nodules if examined with a lens. In some instances the colonies presented a prominent centre with a lobulated margin and appeared as rosettes. A characteristic of the colonies was that they sent into the agar root-like projections. In aërobic agar slant cultures no growth or a slow and very feeble growth was obtained. In stab cultures the growth was sometimes limited to the lower portion of the line of inoculation or was more vigorous there. In bouillon, after three to five days, growth appeared as small white flakes, partly floating and partly collected at the bottom of the tube. Growth occurred in bouillon under aërobic conditions, but was better under anaërobic conditions. The micro-organism in smear preparations from agar cultures appeared chiefly as short homogeneous, usually straight, but also comma-like or bowed rods, whose length and breadth varied. In many cultures short clump rods predominated, and in others longer, thicker, or thinner individuals were more numerous. The ends of the rods often showed olive or ball-like swellings. Some twenty guinea-pigs and rabbits were inoculated, most of them in the peritoneal cavity, with pieces of agar culture. Eighteen animals were killed after four to seventeen weeks, and four were still alive seven to nine months after the inoculation. Seventeen rabbits and one guinea-pig showed at the autopsy tumor growths mostly in the peritoneal cavity and in one instance in the spleen. In the four animals still living tumors were to be felt in the abdominal wall. The tumors in the peritoneal cavity were millet-seed to plum size, and were situated partly on the abdominal wall and partly on the intestines, the omentum, the mesentery, and in the liver or in adhesions. While the surface of the smaller tumors was always smooth, the surface of the larger tumors showed small hemispherical prominences, giving them the

appearance of conglomerates of smaller tumors. On section the larger tumors presented a tough capsule from which anastomosing septa extended inward enclosing cheesy masses. Microscopic examination of the tumors showed in all cases but one the presence of typical actinomyces colonies, in most cases with typical "clubs." The general histological appearance of the tumors was like that of actinomycotic tissue.

Wolff in a later paper reports that an animal inoculated in the peritoneal cavity with a culture of the same organism had lived a year and a half. At the autopsy several tumors were found in the peritoneal cavity, and in the liver a large typical tumor in which were many colonies which by microscopic examination were shown to be typical club-bearing actinomyces colonies.

Naked-eye Appearance of Colonies of Parasite in Tissues.—In both man and animals they can be readily seen in the pus from the affected

FIG. 131

A typical "club"-bearing colony of actinomyces. × 325 diameters. (From Wright.)

regions as small, white, yellowish or greenish granules of pinhead size (from 0.5 to 2 mm. in diameter). When pus has not formed they lie embedded in the granulation tissue.

Microscopic Appearance.—Microscopically these bodies are seen to be made up of threads, which radiate from a centre and present bulbous, club-like terminations (Fig. 131). These club-like terminations are characteristic of the actinomyces. They are generally arranged in pairs, closely crowded together, and are very glistening in appearance. The threads which compose the central mass of the granules are from 0.3μ to 0.5μ in diameter; the clubs are from 6μ to 8μ in diameter.

The organism is *stained* with the ordinary aniline colors, also by Gram's solution; when stained with gentian violet and by Gram's method the threads appear more distinct than when stained with methylene blue. The clubs lose their stain by Gram's method and take the contrast stain.

Isolation of Actinomyces.—Wright[1] recommends that granules, preferably obtained from closed lesions, are first thoroughly washed in sterile water or bouillon and then crushed between two sterile glass slides. In bovine cases make sure the granule has filamentous masses, for if not no culture will grow. The crushed granule is transferred to a tube of melted 1 per. cent. glucose agar at 40° C. The material is thoroughly distributed by shaking and the tube placed in the incubator. A number of granules after washing should be placed on the inside of a sterile test-tube and allowed to dry. In this way, should the material be contaminated, the drying of the granules for several weeks may kill off the other bacteria. The tube should be examined daily. If a number of living filaments were added to the agar a large number of colonies will develop. These will be most numerous in the depth in a zone five to twelve millimetres below the surface.

From this primary culture a colony is cut out and the bit of agar washed in bouillon and then inserted in a tube of melted agar. The growth in this will give material for transplants.

Is actinomycosis due to a single micro-organism or to a group of organisms having widely different characteristics?

Wright[2] has recently made an important research study on this question. His conclusions were as follows: From thirteen human cases and from two in cattle the organisms seem to be all of one species, for the differences among the various strains are no greater than among various strains of tubercle or diphtheria bacilli.

This micro-organism grows well only in agar and bouillon cultures and in the incubator; in the other usual culture media and at room temperature it grows only very little or not at all. It is essentially an anaërobe. It does not form spore-like reproductive elements. In cultures its colonies are similar in character to colonies of the micro-organism in the lesions of actinomycosis. If colonies of the micro-organism are immersed in animal fluids, such as blood serum and serous pleuritic fluid, the filaments of the colonies in immediate contact with the fluid may, under certain unknown conditions, become invested with a layer of hyaline eosin-staining material of varying thickness, and the filament may then disappear. Thus structures are produced that seem to be identical with the characteristic "clubs" of actinomyces colonies in the lesions.

Inoculation experiments on animals were made with the cultures of the micro-organism from thirteen cases, including the two bovine cases. All of these strains were found to be capable of forming the characteristic "club"-bearing colonies in the tissues of the experimental animals. These colonies were either enclosed in small nodules of connective tissue or were contained in suppurative foci within nodular tumors made up of connective tissue in varying stages of development. With the cultures from most of the cases nodular lesions identical in histological character with those of actinomycosis were produced in inoculated animals and

[1] Journal of Medical Research, May, 1905. [2] Loc. cit.

with some of the cultures relatively extensive lesions, considering the size of the animal. The most extensive lesions showed little progressive tendency, and in only a very few instances did multiplication of the micro-organism in the body of the inoculated animal seem probable. In view of the negative or ambiguous results of those who have inoculated healthy animals with actinomyces directly from the lesions, it would seem that the results of the inoculation of animals with the cultures described in this paper afford as much proof as can be expected from such experiments that the micro-organism in the cultures was identical with the micro-organism in the original lesions.

I do not accept the prevalent belief, based on the work of Bostroem, Gasperini, and others, that the specific infectious agent of actinomycosis is to be found among certain branching micro-organisms, widely disseminated in the outer world, which differ profoundly from actinomyces bovis in having spore-like reproductive elements. I think that these should be grouped together as a separate genus with the name of nocardia, and that those cases of undoubted infection by them should be called nocardiosis and not actinomycosis. The term actinomycosis should be used only for those inflammatory processes the lesions of which contain the characteristic granules or "drüsen." That a nocardia ever forms these characteristic structures in lesions produced by it in man or cattle has not been convincingly shown.

Because the micro-organism here described does not grow well on all the ordinary culture media and practically not at all at room temperature, I do not believe that it has its usual habitat outside of the body. It seems to me very probable that it is a normal inhabitant of the buccal cavity and gastrointestinal tract.

The cultures are quite resistant to outside influences; dried, they may be kept for a year or more; they are killed by an exposure of five minutes to a temperature of 75° C.

Occurrence in Animals.—Actinomycosis is quite prevalent among cattle, in which it occurs endemically; it is more rare among swine and horses, and is sometimes found in man. The disease is rarely communicated from one animal to another and no case is known where a direct history of human contagion has been obtained. The cereal grains, which from their nature are capable of penetrating the tissues, have been repeatedly found in centres of actinomycotic infection. This usually occurs in the vicinity of the mouth, where injuries have been accidentally caused. The micro-organism may also be introduced by means of carious teeth. Cutaneous infection has been produced by wood splinters, and infection of the lungs by aspiration of fragments of teeth containing the fungus. The presence of the micro-organism in cereal grains, which was formerly accepted, is denied by Wright and therefore certainly placed in doubt. The further distribution of the fungus after it is introduced into the tissues is effected partly by its growth and partly by conveyance by means of the lymphatics and leukocytes. Not infrequently a mixed infection with the pyogenic cocci occurs in actinomycosis.

In the earliest stages of its growth the parasite gives rise to a small granulation tumor, not unlike that produced by the tubercle bacillus, which contains, in addition to small round cells, epithelial elements and giant cells. After it reaches a certain size there is great proliferation of the surrounding connective tissue, and the growth may, particularly in the jaw, look like, and was long mistaken for, osteosarcoma. Finally, suppuration occurs, which, according to Israel, may be produced directly by the fungus itself.

The experimental production of actinomycosis in animals has been followed by negative or very unsatisfactory results. When artificially introduced into the tissues the organism is either absorbed or encapsulated. If introduced in large quantities multiple nodules are apparently formed in some cases, which may suggest the production of a general infective process; but on closer inspection of these nodules the thread-like portion of the fungus is found to have disappeared, leaving only the remains of the club-like ends, thus showing that no growth has taken place. Ponfick, Johne, Rotter, Lüning, and Hanan claim to have obtained positive results in animals, but according to Bostroem these results are not conclusive. The animals used for experimentation have been calves, swine, dogs, rabbits, and guinea-pigs, the places of inoculation being the anterior chamber of the eye, the subcutaneous intercellular tissue, the peritoneum, and the blood, and the material employed for inoculation being pus from the infected regions in animals and man, very rarely cultures.

Streptothrix Infections.—From widely scattered localities and at long intervals of time reports have been published describing unique cases of disease produced by varieties of micro-organisms belonging to the genus streptothrix. In some of these cases points of similarity can be recognized in the clinical symptoms and the gross pathological lessons, while others differ widely in both respects. They have been found in brain abscess, cerebrospinal meningitis, pneumonic areas, and in other pathological conditions. Eppinger injected cultures into guinea-pigs and rabbits, and observed that it caused a typical pseudotuberculosis. Consolidation of portions of both lungs, thickening of the peritoneum, and scattered nodules resembling tubercles, were noted in a case of human infection as due to a streptothrix by Flexner, in which the pathological picture of the disease resembled so nearly tuberculosis in human beings that the two diseases could be separated only by the causative micro-organism in each case. But in no two cases reported up to the present time have the descriptions of the micro-organisms found agreed in all particulars. In some cases no attempt at cultivation was made. In other cases numerous and careful plants on various culture-media failed to develop the specific organism. In the remaining cases in which the streptothrix was obtained in pure culture, the descriptions of the growth characteristics essentially differ. In a recent review of the literature Tuttle was able to find the reports of only twelve cases in which a streptothrix was found in sufficient abundance to have been an important, if not the

principal, factor in producing disease. These cases were all fatal, and only once was the character of the disease recognized during life. As the clinical symptoms and the lesions in the human subject as well as in the animals experimentally inoculated with the streptothrix often resemble those of miliary tuberculosis, so that a number of these cases have been reported as pseudotuberculosis, the question is naturally suggested whether such cases of streptothrix tuberculosis are not more numerous than the few reported cases would indicate. The almost universal prevalence of genuine tuberculosis and the extreme gravity of the disease have so long occupied the attention and study of the medical profession that much is taken for granted, and cases in which the symptoms and lesions resemble with some closeness those characteristic of the well-known disease may easily be set down without question to the account of the tubercle bacillus. The cases of streptothrix tuberculosis so far reported have all been fatal, and the lesions for the most part have been widely distributed, but in a number of cases old lesions have been found which suggest that the disease may have been localized for a longer or shorter time, and then, by some accident, may have become rapidly general. In this respect, also, these cases may resemble tuberculosis. Whether all cases of streptothrix disease in the human subject are general and fatal, or, as in tuberculosis and actinomycosis, there may be cases of localized disease which recover, are questions which have not been decided at the present time. The methods employed to demonstrate the presence of tubercle bacilli render the streptothrices invisible. Again, unless the observer keeps in mind the possibility of streptothrix infection, he may not appreciate the importance of the slender threads with or without branches, and may consider them accidental bacilli, or varieties of leptothrix or non-pathogenic fungi. As the lungs have appeared to be the seat of the primary infection in most of the cases of human streptothrix disease, it is very desirable that all cases presenting the physical signs of tuberculosis, in which repeated examinations fail to discover the tubercle bacillus, should be systematically examined for streptothrix threads. In this way alone can the frequency of the disease be determined. Gram's method of staining or the Ziehl-Neelson solution decolorized with aniline oil seem to be the most reliable agents for demonstrating these organisms. The streptothrices are widely distributed and are not very infrequently met with, but as yet, with the exceptions mentioned above, very little is known about them. Kruse mentions nineteen varieties, including the actinomyces. Some of them are non-pathogenic; some are pathogenic for certain animals, and others are pathogenic for both man and animals.

In studying the descriptions of the different varieties of these microorganisms, it seems that, as in the case of certain bacteria, different observers may possibly have described the same variety under different names.

Tuttle's report of the case of general streptothrix infection at the Presbyterian Hospital, gives such a good clinical, bacteriological, and

pathological picture of a case of this infection that a considerable portion of it is repeated here:

Six days before her admission to the hospital her illness began with a severe chill, and fever, and pain in her left side and back. The following day the pain in the side was worse and breathing was difficult. She began to cough and had some expectoration, but no blood was noticed in the sputa. At irregular intervals she had alternating hot and chilly sensations.

On admission, the patient complained of pain in the left side of the chest, cough, fever, weakness, and prostration. Her temperature was 103°, and her pulse and respirations were rapid.

Physical Examination.—The patient is poorly nourished and anæmic. Lungs: Anteriorly the right lung is normal. On the left side, over an area two inches by three inches just to the left of the nipple, there is marked dulness to percussion, and bronchial voice and breathing. No rales are heard. Posteriorly, the left lung is normal. At the extreme base of the right lung there is slight dulness and diminished breathing, with crepitant rales after coughing. The voice is normal.

The history of the disease and the physical signs indicated an attack of acute lobar pneumonia, the area of consolidation being small and situated in the lower part of the left upper lobe in front. Frequent and violent coughing, with almost no expectoration, pain in the affected side and in the lumbar region, restlessness and sleeplessness, and involuntary urination were the symptoms noted during the first four days in the hospital. The pneumonic area increased somewhat, and extended backward to the posterior axillary line, and the temperature was continuous at 103° to 103.5°. On the fifth day the temperature fell two degrees, and signs of resolution appeared in the consolidated area. The apparent improvement, however, was of short duration. On the sixth day the temperature rose to 104.5°, and continued to rise each day, reaching 107.5° shortly before death, which occurred on the ninth day in the hospital and the fifteenth day of the disease. During the last four days the patient complained bitterly of pain in the lumbar region and in the thighs and legs, and of intense vesical tenesmus. The stools and urine were passed involuntarily. Signs of consolidation were found in the right lower lobe, behind. There were repeated attacks of profuse sweating. On the day before her death three indurated swellings beneath the skin were noticed. One, on the left forearm, about the size of a walnut, apparently contained pus. Two, of smaller size, were situated in the right groin.

Blood cultures from a vein in the arm, taken on the sixth day, remained sterile. Subsequent attempts failed on account of the feeble circulation. The leukocyte count on the seventh day was 36,000.

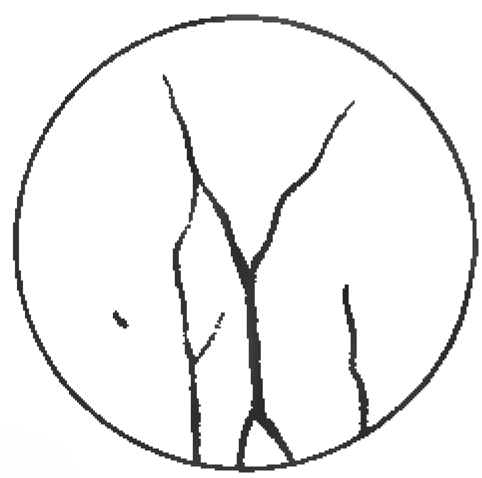
Autopsy.—On the right arm, the left forearm, the abdominal wall, and on both thighs there are eight or ten slightly projecting, rounded, fluctuating, subcutaneous swellings from one-half inch to one inch in diameter. The skin over most of these nodules is unaltered, but over the larger ones there is a slight bluish discoloration. The nodules were

found to be collections of bluish-gray, thick, mucilaginous matter, which is very tenacious and can be drawn out into long threads. The pericardium is normal. The valves are normal. In the wall of the right ventricle are several small white areas which look like septic infarctions, and one of larger size in the wall of the left ventricle corresponds with the position of a thrombus, and apparently was the exciting cause of it. Left lung: In the lower part of the upper lobe is an area of consolidation, gray in color and partly resolved. Right lung: There are a few recent pleuritic adhesions. The lower lobe is thickly studded with miliary tubercles, and scattered through the entire lung are suppurating foci. Liver: There is nothing abnormal. The spleen is normal. Pancreas: In and immediately around the gland there are many small abscesses. Kidneys: The description of one applies to both. The surface is everywhere and evenly dotted with minute white spots, which suggest septic emboli rather than tubercles. A few prominent white nodules, from

Fig. 132

Streptothrix from bouillon culture. (From Tuttle.)

Fig. 133



Young streptothrix threads showing terminal buds. (From Tuttle.)

one-quarter inch to one-half inch in diameter, contain thick, tenacious matter (Fig. 134). Section shows that the entire substance of the kidney is densely studded with these minute white granules.

The gross pathological conditions were interpreted as follows: An old tuberculous nodule in the right lung; acute miliary tuberculosis of the right lung and peritoneum; acute lobar pneumonia, affecting the left lung; septic infarctions and pyæmic abscesses of both lungs, heart muscle, both kidneys, pancreas, mesenteric lymph nodes, and subcutaneous connective tissue. The miliary tubercles of the right lung and peritoneum presented the characteristic appearance of genuine tuberculosis. They were minute, hard, gray, almost translucent nodules, while the granules in the kidneys were of an opaque-white or yellowish-white color.

Microscopic Examination.—Smears from the abscesses beneath the skin and on the surface of the kidneys were stained with methyl-blue, carbol-fuchsin, and by Gram's method. The smears resemble those made of tenacious sputum. There is a large amount of mucoid material

containing a considerable number of leukocytes. Occasionally irregularly curved, thread-shaped micro-organisms are found. They vary considerably in length and thickness, and broken and apparently degenerating fragments are seen. The more slender threads are evenly stained, but some fragmentation or beading of the protoplasm can generally be observed. The thicker threads and broken fragments show deeply stained globules and irregular bodies in a faintly visible rod or thread-shaped covering. Some branching threads are observed, but more commonly they are not branching. The fragments seen in the smears, varying in length and thickness and staining properties, convey the impression of a branching organism; the slender, more evenly stained threads being the younger branches, and the thicker, broken, and granular fragments the parent trunks. No other micro-organisms are found in the smears. Sections from the lower lobe of

Fig. 134

Portion of kidney showing minute and large areas of infection.

the right lung, stained with hæmatoxylin and eosin, showed in certain places the identical microscopic appearances which are considered characteristic of tuberculosis. There are distinct tubercles, of recent growth, consisting of epithelioid cells and giant cells. Others have granular or cheesy centres with epithelioid cells and giant cells at the periphery; and some consist of cicatricial connective tissue surrounded by a zone of epithelioid cells containing giant cells. Many sections from the right lung showing the tuberculous lesions were stained in the usual way for tubercle bacilli. Most careful searching by a number of competent examiners failed to discover any tubercle bacilli or any other micro-organisms. Stained by Gram's method, with care not to decolorize too completely, threads like those described in the abscesses are found in great abundance, but rather faintly stained. No threads can be found within the typical tubercles with giant cells, but in the

zones of small cells around them they are seen in great numbers, winding about among the cells and forming a sort of network. In the minute foci of small cells one or two fragments of threads are generally seen, and a moderate number in the small abscesses. In the areas of more diffuse infiltration these threads are abundant. No other micro-organisms can be found except in the pneumonic area of the left lung, where some groups of cocci are seen. The thread-like organisms are also found in this area and in the other foci scattered through the lung.

The staining methods described by Flexner were found to give the best results. The most reliable and the one requiring the least time is a modified Gram's method. The sections stained with aniline-gentian violet are dipped for a short time in a diluted Gram's iodine solution and then treated with aniline oil until sufficient color has been removed. The aniline oil is then washed out with xylol, and the section is mounted in xylol balsam. The other method mentioned by Flexner was found to be less reliable, but gave more beautiful results when successful. The specimens are first stained with dilute hæmatoxylin solution to bring out the nuclei of the cells and then are stained with carbol-fuchsin and decolorized with aniline oil as before. Long staining with carbol-fuchsin and careful treatment with aniline oil are necessary for success. Many times the micro-organisms were completely decolorized by this method, but when successful the dark-red threads winding among the bluish nuclei produced a striking picture.

Culture Experiments.—Six tubes of Loeffler blood serum were inoculated from the kidneys. The tubes were placed in the incubator. On the third day after inoculation minute white colonies appeared in some of the tubes, and on the fifth day all the tubes showed from three to ten or twelve similar colonies in each. The colonies increased in size until some of them reached a diameter of one-eighth of an inch, but most of them were smaller. The color, at first white, changed to yellowish-white and then to a decided pale yellow. Having attained a certain size at the base, the colonies ceased to extend, but became more and more prominent. The growth was apparently more rapid at the periphery, and the fully developed colony was round, with convex sides and with a cup-shaped depression at the top. The height of the colony was sometimes greater and sometimes less than the diameter of the base. The well-developed colonies cling firmly to the surface of the medium and are not easily detached or broked up. The growths in all of the tubes were absolutely pure, and consisted of branching threads like those found in the sections. A more minute description of these organisms will be given below.

Transplants on Loeffler blood serum produce a pale sulphur-yellow growth, forming a layer with a slightly irregular and wrinkled surface and prominent edges where the growth continues longest. The color remains the same until the medium dries, when it becomes white. The growth clings so tenaciously to the surface of the medium that in removing a specimen for examination a portion of the blood serum also is torn away.

Loeffler blood serum seems to be the most suitable medium for cultures. The growth on this medium is more rapid and abundant than on any of the other media tried.

On plain agar and glycerin agar the growth is the same as on blood serum, but is less rapidly developed.

In bouillon the growth is slow and takes place only at the surface and on the sides of the tube. The bouillon remains perfectly clear and no pellicle or scum develops on the surface. If the tube is not disturbed or jarred, minute white tufts are seen clinging to the surface of the glass. But if the tube is shaken even slightly they sink slowly to the bottom, forming a white, fluffy layer. These growths when undisturbed resemble minute balls of thistle-down. The yellow color is not apparent even in the mass at the bottom of the tube.

It is strictly aërobic. In sealed tubes a very scant growth is obtained, but when deprived of oxygen absolutely no growth can be detected, although life is preserved for long periods. Cultures from a tube kept for two months without oxygen, and showing no sign of growth, developed rapidly when exposed to the air.

Morphology.—The relative thickness of the threads varies somewhat in different parts of the same individual branching organism, and considerably in specimens taken from different culture media. If the growth is rapid and luxuriant, the threads are thicker than in specimens taken from growths on less suitable media. For instance, when grown on blood serum the threads are comparatively thick and coarse, but those growing in bouillon are very slender and delicate. The main trunk also is often thicker than the branches. When unstained they are homogeneous gray threads, without any appearance of a central canal or double-contoured wall. There is never any segmentation of the threads. When properly stained there is always a distinct beading or fragmentation of the protoplasm, but overstaining with fuchsin produces rather coarse, evenly red rods. The branching is irregular and without symmetry, and the branches are placed at a wide angle, very nearly, and sometimes quite, at right angles. This is best seen in specimens taken from liquid media. The irregularly stellate arrangement of the branches, which was observed by Eppinger in his original specimen, is often seen in young organisms floated out from a liquid medium. Eppinger considered this form sufficiently characteristic to warrant the term asteroides to distinguish the species. But terms like asteroides and arborescens do not sufficiently distinguish the species of a genus which is characterized by more or less tree-like branching, and so far no satisfactory nomenclature has been adopted.

Spore Formation.—On examining the deep-orange or red-colored growth upon potato, one is surprised to find that the threads have entirely disappeared, and that the specimen consists of moderately large cocci. In very young cultures upon potato both threads and cocci are found, but the relation between them cannot be seen in smears prepared in the ordinary way. In older cultures no trace of the thread can be found, unless some fine granular matter may represent them

These cocci represent the spore form of the organism, and when planted upon blood serum the branching threads again appear. The spores stain readily with carbol-fuchsin and are not easily decolorized. They are spherical, or nearly so, but often appear somewhat elongated, apparently from beginning germination. They are killed by exposure to moist heat of 65° to 70° C. for an hour, but are more resistant to dry heat. Exposure upon a silk thread, to a temperature above 120° C. for an hour and a half did not affect their vitality. The last sixty minutes of this time was at 125° to 127° C., and the bulb of the thermometer was within the tube in contact with the thread. No growth was obtained after an hour's exposure at 135° C. Drying destroys the threads after a comparatively short time, but the spores retain their vitality for an indefinite period. A dried-up potato culture which was planted March 1, 1900, and has stood in the laboratory ever since, still retains its vitality at the end of almost four years. Plants made from this specimen upon blood serum show an abundant yellowish growth at the end of twenty-four hours.

The germination of the spores and the growth of the threads were observed under the microscope in a hanging drop in the chamber of a hollow slide. Here all stages of growth may be seen, from the elongated spore to the fully developed branching organism. The spore sends out a delicate sprout which very early looks like a bacillus with a bulbous extremity. The sprout soon branches, and the growth proceeds rapidly with frequent branching in all directions. Slight clubbing of the ends of the branches is often seen, but generally the ends are not club-shaped. In a few stained specimens an extremely delicate, almost colorless, club-shaped structure was seen at the ends of the branches. This appearance was not constant, and possibly was produced artificially. It can be seen in some of the photographs.

The development of the spores was not so easily demonstrated. In specimens taken at frequent intervals from potato cultures the threads become more and more broken up into short fragments, and the number of free spores increases very rapidly. Some of the fragments appear to have a spore at each end, and some have three or more spores. The free spores show no regular arrangement, but occasionally a short chain of five or six can be found.

The identity of this micro-organism is not fully established. It is undoubtedly a streptothrix, but it does not agree in all particulars with any of the varieties described. It approaches most nearly Eppinger's variety, but at no stage of its growth is it ever motile. The color and character of the growth upon different media and the sporulations upon potato agree with the descriptions of the streptothrix Eppingeri.

Animal Inoculations.—A number of rabbits and guinea-pigs were inoculated subcutaneously upon the abdomen and in the neighborhood of the cervical, axillary, and inguinal lymph nodes with colonies broken up in salt solution. Indurated swellings were produced at the point of inoculation and a number of abscesses resulted. The abscesses developed rapidly and some of them opened spontaneously, while

others were incised. The material evacuated did not resemble ordinary pus, but was thick and mucilaginous and exceedingly tenacious, like that from the subcutaneous abscesses of the patient described above. The microscopic appearance was the same, and the streptothrix threads were found in considerable numbers. Pure cultures of the streptothrix were easily obtained from the pus whether the abscesses ruptured spontaneously or were incised. Several rabbits and guinea-pigs and two cats received peritoneal inoculations, but none of them showed any sign of infection. Sometimes at the point of inoculation a few tuberculous nodules were found at autopsy, but cultures were not obtained from them. No local infection of any consequence and no general infection was produced in this way. Thus far little virulence had been shown by the streptothrix in inoculation experiments; but when rabbits were inoculated intravenously, a rapidly fatal general infection was produced, and the lesions were similar in kind and distribution to those described in the human subject. Other cases reported are the following:

Ferré and *Faguet* found in Bordeaux, in a cerebral abscess in the centrum ovale, a branching fungus, colored by Gram, which corresponded to the streptothrix. It grew on agar in round, ochre-colored colonies; on potato there was little growth visible; slimy, tough colonies, which became gray and remained free from white dusting on the surface. Inoculations in rabbits and guinea-pigs were negative.

CLADOTHRIX AND STREPTOTHRIX IN CASES SIMULATING ACTINOMYCOSIS OR TUBERCULOSIS. INTERMEDIATE CASES BETWEEN STREPTOTHRIX AND ACTINOMYCOSIS.—*Gasten* found in a case of apparently typical actinomycosis, in which abscess cavities were found along the spinal column, not the usual actinomyces in the yellow, granular pus, but a fine mass of filament. Cultures grew on all the ordinary media, best at incubator temperature, but also at lower temperature on gelatin. The gelatin stick culture, which was especially characteristic, formed on the surface a whitish button; delicate thread stretched out in all directions from the point of inoculation. On agar and potato rumpled, folded films with white deposit on the surface, which contained spores. Animal inoculation gave positive results only in a few cases of intraperitoneal injection of rabbits and guinea-pigs. Purulent nodules were found in the peritoneum. Gasten called the organism "cladothrix liquefaciens."

Sabraces and *Rivière* found, in a case of cerebral abscess and a case of chronic lung disease with occurrence of subacute abscesses, fungi which differed from actinomyces. The organisms were contained in the lungs and pus in the latter in pure culture. They grew best at 37° C. in the presence of oxygen. On agar plates round, wart-like colonies were found with yellowish under and whitish upper surface. Grew particularly well on fat and glycerin media; in milk a flesh-colored rim was developed; in glycerin agar a rough, brownish deposit, becoming black with age. Gelatin was liquefied. The culture had a strong odor of old mould. A yellowish pigment was usually produced which dis-

solved in ether. In an atmosphere of pure oxygen a brown pigment. Animal experiments gave positive results only when to a fourteen-day-old bouillon culture lactic acid was added; then pseudotuberculosis was produced.

Eppinger found in post-mortem examination of a case of chronic cerebral abscess, which was the result of purulent meningitis, in the pus and abscess walls, etc., a delicate fungoid growth which he succeeded in cultivating on various media. On sugar agar it formed yellow, rumpled colonies which finally developed into a skin. On potato it grew rapidly, but the colonies remained small, at first a white, granular deposit, which afterward turned red, and on the twentieth day resembled a crystallized almond. It did not grow well on gelatin. In bouillon it formed on the surface a small white granule, which became deeper in the centre as it grew and sunk to the bottom as a white deposit. The bouillon remained clear.

Microscopically the fungus consisted of fine threads without branches, which exhibited distinct motility. No flagella were observed. It was judged to be a cladothrix, to which the name "asteroides" was given by the author. It proved to be quite pathogenic for rabbits and guinea-pigs, and produced an infection of pseudotuberculosis. Mice were not affected by inoculation.

Numerous cases have since been observed in which the streptothrix proved to be the cause of chronic lung diseases, clinically suspected to be tuberculosis.

CHAPTER XXXIV.

THE PATHOGENIC FUNGI AND YEASTS (BLASTOMYCETES)—DISEASES DUE TO MICRO-ORGANISMS NOT YET IDENTIFIED.

The Fungi.

Most of the fungi are not pathogenic and interest us merely as organisms which are apt to infect our bacteriological media. Some are, however, true parasites, and already we know that ringworm, favus, thrush, and pityriasis versicolor are caused by fungi. Only those causing ringworm, favus, pityriasis, and soor will be touched on.

Trichophyton (Ringworm Fungus).

Ringworm of the body or hairless parts of the skin, *tinea circinata*, and ringworm of the hairy parts, *tinea tonsurans* and *tinea barbæ* or *tinea sycosis*, are due to the fungus *trichophyton*, discovered by Gruby in the human hair, and between the epidermal cells by Hebra, and obtained in free cultures by gravity.

Fig. 126

Hair riddled with ringworm fungus. Megalosporon variety.

According to Sabouraud, whose conclusions are based on an extensive series of microscopic examinations of cases of tinea in man and animals, of cultivation in artificial media, and of inoculation on man and animals, there are two distinct types of the fungus trichophyton causing ringworm in man—one with small spores (2 to 3 mm.) which he calls "T. microsporon," and one with large spores (7 to 8 mm.) which he calls "T. megalosporon." They differ in their mode of growth

on artificial media and in their pathological effects on the human skin and its appendages. T. microsporon is the common fungus of tinea tonsurans of children, especially of those cases which are rebellious to treatment, and its special seat of growth is in the substance of the hair. T. megalosporon (Fig. 135) is essentially the fungus of ringworm of the beard and of the smooth part of the skin; the prognosis as regards treatment is good. One-third of the cases of T. tonsurans of children are due to trichophyton megalosporon. The spores of T. microsporon are contained in a mycelium; but this is not visible, the spores appearing

FIG. 136

These two half-plates show three months' growth on peptone-maltose agar of two megalosporon varieties of the ringworm fungus. Natural size.

irregularly piled up like zoöglœa masses; and, growing outside, they form a dense sheath around the hair. The spores of T. megalosporon are always contained in distinct mycelium filaments, which may either be resistant when the hair is broken up or fragile and easily breaking up into spores. The two types when grown in artificial cultures show distinct and constant characters. The cultures of T. microsporon show a downy surface and white color; those of T. megalosporon a powdery surface, with arborescent peripheral rays, and often a yellowish color. Although the morphological appearances, mode of growth, and clinical effects of each type of trichophyton show certain characters in general,

yet there are certain constant minor differences which point to the fact that there are several different kinds of species of fungus included under each type. The species included under T. microsporon are few in number, and, with the exception of one which causes the common contagious "herpes" of the horse, almost entirely human. The species of T. megalosporon are numerous and fall under several natural groups, the members of which resemble one another both from clinical and mycological aspects (Fig. 136). Many animals are subject to the growth upon their skins of particular species of T. megalosporon.

Achorion Schoenleinii (Favus).

Favus is due to a fungus discovered by Schoenlein in 1839, and called by Remak *Achorion schoenleinii*. The disease is communicated by contagion, the fungus being often derived from animals, especially cats, mice, rabbits, fowls; and dogs are also subject to it. It grows much more slowly than the ringworm fungus, and is, therefore, not so easily transmitted. Want of cleanliness is a predisposing factor. The fungus seems to find a more favorable soil for its development on the skin of persons in weak health, especially from phthisis, than in others.

FIG. 137

A portion of a favus-infected hair; magnified.

Pathologically, the disease represents the reaction of the tissues to the irritation caused by the growth of the fungus. The spores generally find their way into the hair follicles, where they grow in and about the hair (Fig. 137). The favus fungus grows in the epidermis, the density of the growth causing pressure on the parts below, thus crushing out the vitality of the hair and giving rise to atrophic scarring. The disease shows a marked preference for the scalp, but no part of the skin is exempt, and even the mucous membranes are liable to be attacked. Kaposi has reported a case in which a patient suffering from universal favus died, with symptoms of severe gastrointestinal irritation, which was found after death to be due to the presence of the favus fungus in the stomach and intestine. On the scalp it first appears as a tiny sulphur-yellow disk or *scutulum*, depressed in the centre like a cup and pierced by a hair. This is the characteristic lesion. The cup shape is attributed

by Unna to growth at the sides proceeding more vigorously than at the centre.

There is some difference of opinion as to whether there is only one or several varieties of favus fungus. It was suggested by Quincke that there are three different species of favus fungus. Later investigations have apparently shown, however, that the achorion Schoenleinii is the only fungus of favus.

FIG. 138

Five-months-old colony of favus on peptone-maltose agar; actual size.

The favus fungus is readily cultivated at the body temperature, and also at room temperature, in the ordinary culture media, as agar, blood serum, gelatin, bouillon, milk, infusion of malt, eggs, potato, etc. (Fig. 138). The growth develops slowly and shows a preference to grow beneath the surface of the medium—except on potato, upon which it develops on the surface in layers. The characteristic form of growth is that of moss-like projections from a central body. The color is at first grayish-white, then yellowish. As seen under the microscope, ray-like mycelium filaments are developed, which divide into branches. The ends are often swollen or club-shaped, and there are various enlargements along the body of the filament.

Pityriasis Versicolor.

This organism belongs to a group of fungi which, in contrast to the more parasitic fungi, favus and trichophyton, invades only the most superficial layers of the skin. It does not penetrate the deeper layers nor does it give rise to any considerable pathological changes in the skin or hair. Although the vegetative elements of these fungi are much more numerous in the affected portions of skin than is the case with the more parasitic species, they are not nearly as contagious as the latter.

By preference pityriasis versicolor attacks the chest, abdomen, back, and axillæ; less frequently neck and arms, while exceptionally it attacks also the face. The growth shows itself as scattered spots varying in color from that of cream-coffee to reddish-brown. The spots are readily scraped off and show fine lamellation or scaling. Occasionally the spots are confluent, and sometimes arranged in ring form like *herpes tonsurans*.

In spite of their slight contagiousness this is one of the most frequent dermatomycoses. Although it is distributed widely over the earth, it is more frequently observed in southern than in northern countries.

Persons with a tender skin and a disposition to perspire freely are particularly affected by pityriasis versicolor, and this is undoubtedly the only reason why the affection is so frequently observed in consumptives.

Women are more frequently attacked than men, while children and old people are rarely affected.

The source of infection is unknown, since the absence of contagion has frequently been demonstrated. It seems likely that the spores of this fungus are so widely distributed that susceptible individuals are easily infected.

The arrangement of the fungus in the scales of epidermis is characteristic. The short and thick-curved hyphæ (7μ to 13μ long and 3μ to 4μ wide) surround large clumps of spores. The spores are coarse, doubly contorted (4μ to 7μ) or round. On staining with Ziehl's solution the spores are seen to contain deeply stained globules lying, in all probability, on the inner surface of the cell membrane. The rest of the protoplasm is but little stained, or not at all. One frequently finds that these globules have disintegrated into numerous fine granules. The globules are also found free; what their nature is does not appear; they are not found in cultures, the freshly developed spores showing only a single globular mass of protoplasm possessing a fine blue lustre.

Soor Fungus (Thrush).

Soor, as is well known, occurs most frequently in the oral mucous membrane of infants during the early weeks of life. It is also found as a slight mycosis in the vagina, especially of pregnant women. In rare cases the disease attacks adults, and then especially those whose system has been undermined by other diseases, such as diabetes, typhoid patients, etc. A few cases are recorded in the literature in which this fungus has given rise to constitutional disease. In these cases autopsy has shown abscesses in various parts of the body, such as in the lungs, spleen, kidney, and brain.

In the lesions of the disease as well as in cultures, this fungus appears both as an yeast and a mycelium. The yeast cells are oval in form, about 5μ to 6μ long and 4μ wide, and can in no way be distinguished from other yeast cells either by their appearance or their method of propagation. The threads of the mycelium vary very much in length and thickness, and show all intermediate forms between a typical and a budding mycelium.

Soor is not much influenced by acids or alkalies, growing well both in acid and in alkaline media. On the other hand, it is very susceptible to the common disinfectants, especially salicylic acid, corrosive sublimate, phenol, etc. This fact is made use of in local treatment.

Blastomycetes (Yeasts).

These micro-organisms are of the greatest importance in brewing and baking, but as yet no important pathological lesions in man have been attributed to them, although certain recent experiments have shown that some varieties when injected are capable of producing tumors and many are pathogenic for mice. They are not uncommonly

present in the air and in cultures made from the throat. They consist of round or oval cells, usually many times larger than the bacteria. They usually reproduce themselves by budding, a portion of the protoplasm budding and finally being cut off to form a new individual.

For many centuries blastomycetes, or yeasts, have proven themselves to be of great benefit to man, untold millions of them being used daily in breweries, distilleries, and other industries. Until a few years ago this group of organisms stood alone among other allied forms of life as being the only one in which pathogenic species were unknown. It is not more than ten years since the discovery of the first of the disease-producing yeasts. Since that time these organisms have been studied not only because of their interesting biological and physiological characteristics, but also from the point of view of the physician and etiologist. Our present knowledge concerning the pathogenic yeasts may be briefly summarized as follows:

The position which the yeasts occupy in systematic biology (botany) has not, thus far, been accurately determined. In fact, it is even doubted whether they constitute independent fungi or are perhaps a particular form of growth of more highly organized fungi, especially of the mould fungi. This hypothesis was formulated by Brefeld about thirty years ago, but has not thus far been proved. For the present it seems advisable to retain the yeasts in a group of fungi by themselves.

The chief characteristic of the yeasts is their peculiar method of reproduction which in most cases is by means of budding. For this reason these organisms go by the name of *blastomycetes* in contrast to the fission fungi, or *schizomycetes*, and the mould fungi, or *hyphomycetes*. A transition between the blastomycetes and the hyphomycetes is formed by the *oidien*, which at one time grow to long threads, at another time (under certain conditions almost exclusively) multiply by budding. But no hard-and-fast line exists between these classes, for the yeasts can at times develop short hyphæ, at other times, in rare cases, form new individuals by segmentation.

The most important property of yeasts, though one not possessed by all to the same degree, is that of producing alcoholic fermentation. In practice we distinguish between the yeasts that can be employed practically, "culture yeasts," and those which often act as disturbing factors, so-called "wild" yeasts.

The shape of most of the culture yeasts is oval or elliptical (Fig. 139). Round or globular forms are more often met with among the wild species and such as excite only a slight degree of fermentation. They are known as "torula" forms. But sausage-shaped and thread forms are also met with.

The individual yeast cells are strongly refractive, so that under the microscope at times they have almost the lustre of fat droplets. This is important because in examining fresh tissues the yeast cells may be hard to distinguish from fat droplets, often requiring the aid of certain reagents for their identification.

The size of the individual yeast cells varies enormously, even in

those of the same species or the same culture. In old colonies individuals may be found hardly larger than cocci, 1 to 2μ, while in other colonies, especially on the surface of a liquefied medium, giant yeast cells are found often attaining a diameter of 40μ or more. In spite of these wide fluctuations, however, the various species are characterized by a fairly definite average in size and form.

During the process of budding the nucleus of the cell moves toward the margin, where it divides. At this point the limiting membrane of the cell ruptures or a hernia-like protrusion develops which has the appearance of a button attached to the cell. The daughter-cell so formed rapidly increases in size and gradually assumes the shape and size of the mother-cell.

A fact of the utmost importance for the propagation of the blastomycetes and continuation of the species is the formation of spores. In this also the cell nucleus takes part, dividing into several fragments,

FIG. 189

Saccharomyces Busse. × 360 diameters. (From Kolle and Wassermann.)

each of which becomes the centre of a new cell lying within the original cell. These new cells possess a firm membrane, a cell nucleus, and a little dry protoplasm. The number of spores developed in the yeast cells varies, but is constant for a given species. As a rule, one cell does not produce more than four endogenous spores, so-called astrospores; but species have been observed—*e. g.*, *schizosaccharomyces octosporus* (Beijerinck)—in which eight spores are found.

The vitality of yeasts is truly enormous. Hansen as well as Lindner were able to obtain a growth from cultures twelve years old. Busse succeeded in getting a luxuriant growth from a dry potato culture seven and a half years old, and almost as hard as bone.

As stated above, the most important property of yeasts is that of producing alcoholic fermentation. While a large number of yeasts are merely able to decompose dextrose into alcohol and carbon dioxide, there are some which ferment cane-sugar, others which invert and ferment starch; in fact, all kinds of carbohydrates may be decomposed.

As was first shown by Buchner, the fermentation is due to enzymes produced by the yeast cells. These enzymes differ in the different species, and hence also, their action differs.

The pathogenic blastomycetes may be briefly summarized as follows:

Saccharomyces Busse, isolated in 1894 by O. Busse from the tibia of a thirty-one-year-old woman, who died thirteen months after the first symptoms appeared. The autopsy showed numerous broken-down nodules on several of the bones, in the lungs, spleen, and kidneys. The yeast was cultivated from all these foci (Fig. 139).

Saccharomyces subcutaneus tumefaciens, isolated in 1895 by Curtis. The patient was a young man showing multiple tumors on the hips and neck having the gross appearance of softened myxosarcomata.

This yeast is pathogenic for rats, mice, and dogs, only slightly so for rabbits, and not at all for guinea-pigs.

Gilchrist has described a case of pseudolupus vulgaris caused by a blastomyces. The disease lasted ten years, during which time many nodules developed on the face, back of the hands, scrotum, thigh, and neck. The nodules ulcerated and then healed, leaving scars. Busse believes that this organism should be classed with oidium, and this opinion is shared by Buschke.

In a case reported by Hektoen in 1899 that author describes skin lesions very similar to those observed by Gilchrist. The organism obtained on cultivation, however, differed somewhat from Gilchrist's. Injected into rats this yeast produced abscesses and caused the death of the animal in five days.

Lundsgaard reported a case of ophthalmia due to an yeast. His patient, a man thirty-four years old, had a severe hypopyon keratitis, in the pus of which many yeasts were present. Pure cultures of the yeast inoculated into guinea-pigs produced abscess both at the site of inoculation and in the lymph glands.

Buschke isolated an yeast from a cervical discharge in which no gonococci were present. The yeast was pathogenic for guinea-pigs.

In 1895 Dr. G. Tokishige reported that an epidemic quite common among horses in Japan, known as "Japanese worm," "benign worm," or "pseudoworm," is caused by saccharomyces. The disease begins in the skin in the form of hard, painless nodules from the size of a pea to a walnut. These break down and give rise to gradually extending ulcers. Pure cultures of the saccharomyces are pathogenic only for horses, not for rabbits, guinea-pigs, or hogs. In the districts where the disease prevails among horses it is also frequently seen in cattle.

Shortly after Tokishige's publication a similar disease occurring in horses in Italy and Southern France was identified as being caused by saccharomyces. Cultures of this yeast however, differ somewhat from that obtained in Japan; so that Busse is inclined to regard the two as two different species of blastomycetes.

In recent years the attempt has been made to connect the development of cancerous growth with blastomycetes. This is due in a meas-

ure to a certain similarity between the yeasts and the cell inclusions or so-called "parasites" of cancer, and, further, to the fact that when yeasts are injected into the animal body tumor-like nodules are often developed at the site of inoculation and in the internal organs. But these nodules are not tumors in the pathological sense of the term, but merely masses of blastomycetes mixed with inflammatory tissue proliferations to a very variable degree. At the present time Sanfelice and his pupils are perhaps the only ones who regard the thickenings produced in the tissues by *Saccharomyces neoformans* as true tumors. His work, however, is not at all convincing.

Diseases in which the Micro-organisms Exciting them are as yet Undetected.

Measles.—Many bacteria as well as bodies supposed to be protozoa have been described by various investigators as occurring on the mucous membranes or in the blood of those sick of measles. None of these have been established as the exciting factor. Hektoen has recently transferred blood from a case of measles to two individuals and so communicated the disease.

Scarlet Fever.—Both streptococci and protozoa have been described as the exciting factors in this disease, as already previously mentioned. The streptococci are certainly present, but are looked upon by most as secondary invaders. They undoubtedly add greatly to the gravity of the disease. The bodies described by Mallory as protozoa are still under investigation. Serum treatment has been used to overcome the streptococcus infection. The best results have been obtained in Vienna, and by Moser. He uses a serum obtained from horses receiving multiple cultures from cases of scarlet fever. Only about one horse in three gives a curative serum. The doses used are very large (100 to 200 c.c.). The results claimed are very striking.

Typhus Fever.—Nothing has as yet been determined concerning the micro-organisms exciting this disease.

Smallpox.—Streptococci as secondary invaders add here, as in scarlet fever, a dangerous infection. The status of protozoa is described fully under the section on Protozoa.

Syphilis.—The bacilli described by Lustgarten and others are now no longer considered as a factor. Schaudinn's recent discovery of spirochæte is considered under the protozoa.

Rabies (Hydrophobia).—No bacteria have been discovered that are considered as factors. The possibility of the negri bodies being protozoa and the exciting factor is considered under Protozoa.

Whooping-cough.—Jochmann and Krause, in Germany, and Wollstein, in this country, have shown that bacilli differing slightly in cultural reactions and in agglutination from typical influenza bacilli can be detected in practically all cases of whooping-cough during the acute stages. Wollstein proved that the blood of cases of whooping-cough agglutinated these bacilli frequently in dilutions of 1:200 and over.

The question is still undecided as to whether these bacilli are anything more than very frequent causes of secondary infection.

Pemphigus Neonatorum.—Several micrococci have been described as the cause of infection.

Impetigo Contagiosa.—The findings have been similar to those in pemphigus.

Scurvy.—This disease is probably not due to micro-organisms.

Mumps.—Diplococci have been considered by several investigators as possibly being the exciting organisms.

Noma.—It is as yet undecided whether this disease is due to one or to several micro-organisms. A special predisposition of the tissues is necessary. A streptothrix, pseudodiphtheria bacilli, and diphtheria bacilli have been the organisms most frequently present.

Articular Rheumatism.—The specific organisms of this disease have been sought in the synovial fluid, blood, vegetations on heart valves, and in exudates on tonsils, etc. Streptococci have been, of all bacteria, most frequently found. They grow in short chains or as diplococci. Most bacteriologists believe the exciting factor has not yet been identified and that the streptococci and other bacteria are secondary infections.

Beriberi.—Micro-organisms, both of bacterial and protozoan nature, have been considered as the exciting factor, but nothing definite has been proven.

Yellow Fever.—The bacillus described by Sanarelli as the exciting factor is now known to be at most a rather frequent secondary invader. Reed, Carroll, and Agramonte have shown that the stegomyia fasciata is the only carrier of the infecting agent. Twelve days after biting a yellow-fever patient the mosquito is able to infect a non-immune by biting. The insect continues for a number of weeks to be capable of infecting man. The blood of yellow-fever patients is only capable of infecting mosquitoes during the first few days. It has also been impossible to infect mosquitoes by letting them bite the body of a person who has died of yellow fever. The blood of yellow-fever patients injected into non-immunes produced the disease. The virus of yellow fever is apparently capable of passing through a Berkefeld filter. At present nothing is known about the micro-organism. The bite of the mosquito is the only known method of causing infection. The clothing and the discharges from the mouth, kidney, and intestines are harmless.

Dengue.—The organism exciting this disease is unknown. An influenza-like bacillus has been observed as frequently present. The mosquito has also been suspected as the carrier of infection.

Invisible Micro-organisms.—There are a number of diseases from which the infectious material passes through stone filters which are known not to allow the passage of visible micro-organisms. The horse sickness of South Africa, yellow fever, and the cattle plague, pleuropneumonia of cattle, are of this nature.

CHAPTER XXXV.

THE BACTERIOLOGICAL EXAMINATION OF WATER, AIR, AND SOIL—THE CONTAMINATION AND PURIFICATION OF WATER—THE DISPOSAL OF SEWAGE.

THE bacteriological examination of water is undertaken with the purpose of discovering whether any pathogenic bacteria are liable to be present. The determination of the number of bacteria in water was for a time considered of great importance, then it fell into disrepute, and the attempt was made to isolate the specific germs of diseases which were thought to be water-borne. At first these attempts seemed very successful in that supposed typhoid bacilli and cholera spirilla were found. Further study revealed that there were common water and intestinal bacteria which were so closely allied to the above forms that the tests applied did not separate them. Even the use of a serum from an animal immunized to injections of the typhoid bacillus was found to agglutinate some other bacteria in high dilutions; so that the test as usually carried out was insufficient. With the latest technique it is probable that the serum from an immunized animal from which the group agglutinins have been absorbed will be sufficient to identify any bacillus which it agglutinates. The practical impossibility of getting typhoid bacilli from suspected water caused a return to the estimation of the number of bacteria in water and above all to the estimation of the number of intestinal bacteria. It is known that the group of colon bacilli have about the same duration of life as the typhoid bacilli, and as the colon bacilli come chiefly or wholly from the intestinal passages of men and animals, it was fair to assume that typhoid bacilli could not occur without the presence of the colon bacillus. The latter could, of course, occur abundantly without the typhoid bacillus.

During the past few years the attention of sanitarians has peen seriously devoted to the interpretation of the presence of smaller or larger numbers of colon bacilli in water, until at present upon the quantitative analysis (measuring, within certain limits, decomposing organic matter) and the colon test (indicating more specifically that derived from intestinal discharges) the bacteriological analysis of water is based.

Technique of Quantitative Analysis.—The utmost care is necessary to get reliable results. A speck of dust, a contaminated dish, a delay of a few hours, an improperly prepared agar or gelatin, a too high or too low temperature, may introduce an error which would make a reliable test impossible.

COLLECTION OF SAMPLES.—The small sample taken must represent the whole from which it was drawn. If a brook-water, it must be taken some distance from the bank; if from a tap, the water in the pipes must

first run off, for otherwise the effect of metallic substances will invalidate the results; if from lake or pond, the surface scum or bottom mud must be avoided, but may be examined separately. The utensils by which the water is taken should be of a good quality of glass, clean and sterile. From a brook the water can be taken directly into a bottle, the stopper being removed while it fills; from a river or pond it can be taken from the bow of a small boat, the bottle being held inverted on a pole with a clamp until it has entered the water; from a well a special apparatus has been devised by Dr. A. C. Abbott, where a bottle with a leaded bottom is so held that when lowered to the proper depth a jerk will remove the cork and allow the bottle to fill. The same device can be rigged up readily by anyone. The sample of water should be tested as soon as possible, for the bacteria immediately begin to increase or decrease. In small bottles removed from the light predatory micro-organisms and many bacteria begin to increase, and among these are the members of the colon group. Thus, the Franklands record a case in which in a sample of well-water kept during three days the bacteria increased from 7 to 495,000; while Jordan found that in a sample the bacteria in forty-eight hours fell from 535,000 to 54,500. In a sample I took from the Croton River the colon bacilli during twenty-four hours increased from 10 to 100 per c.c. The only safe way to prevent this increase is to plate and plant the water in fermentative tubes within the space of one or two hours. It is far better to make the cultures in the open field or in a house rather than to wait six to twelve hours for the conveniences and advantages of the laboratory. If sent to the laboratory, water should be kept below 40° C.

The third matter of great importance is the adding of proper amounts of water to the broth in the fermentation tubes and the media for plating. Usually 1 c.c., 0.1 c.c., and 0.01 c.c. are added to the fermentation tubes and to 10 c.c. of the melted nutrient agar or gelatin. If possible duplicate tests should always be made. When it is desired to know whether colon bacilli are present in larger amounts than 1 c.c., quantities as great as 10 or 100 c.c. can be added to bouillon, and then after a few hours 1 c.c. added to fermentation tubes. Less than ten colonies and more than two hundred on a plate give inaccurate counts, the smaller number being too few to judge an average and the larger number interfering with each other.

The chemical composition of the medium on which the bacteria are grown affects the results of the analysis. Nutrient 1 per cent. agar gives slightly lower counts than gelatin, but on account of its convenience in summer and its greater uniformity it is being more and more generally used for routine quantitative work. There is an optimum reaction for every variety of bacteria, and to ensure uniformity the committee of the American Public Health Association in 1897 adopted a standard which was as near as possible to the average optimum for water bacteria. Such a uniform standard is a necessity to secure comparability of the results of various observers. At best only a certain proportion of bacteria develop, and it is only important that

our counts represent a section through the true bacterial flora which fairly represents the quick-growing sewage forms. Comparability is the vitally essential factor.

The temperature at which the bacteria develop is of great importance, and they should be protected from light. The access of oxygen which prevents the growth of anaërobes must also not be forgotten. As a rule, the plate cultures are developed for three days at room temperature, and for eighteen hours at incubator temperature. Some bacteria do not develop colonies in three days, but these are neglected. The number of bacteria growing at room temperature is usually five to ten times as many as at 35° C.

The glucose broth is placed at 37° C. for the development of the colon bacilli. The fermentation tubes not showing gas are recorded and usually discarded. Those showing gas are suspected to contain colon bacilli. To a number of tubes containing melted litmus-lactose agar at about 44° C. are added 1, 0.1, and 0.01 loop of the culture fluid. Plates are poured and the whole placed in the incubator. The bacillus coli ferments lactose and thus produces acid; so that if colon bacilli are present we have a number of red colonies on a blue field. Later, if many colon bacilli were present, the whole medium becomes acid. At forty-eight hours, on account of alkali being produced, the blue color returns.

If after inspection red colonies are seen, four or five are picked and planted into glucose bouillon and other media. For the characteristics of the colon bacilli the Massachusetts State Board of Health uses six media—gelatin, lactose agar, dextrose broth, milk, nitrate solution, and peptone solution.

Significance of the Colon Bacillus.—The colon test has been received by the majority of engineers and practical sanitarians with great satisfaction, and has been applied with confidence to the examination, not only of water, but of shell-fish and other articles of food as well. On the other hand, some have denied its value. Bacteriologists have found bacilli like certain members of the colon group in apparently unpolluted well-water. The discovery that most animals have colon bacilli apparently identical in the usual characteristics studied with those of man has complicated matters. Thus a fresh hillside stream may be loaded with colon bacilli from the washings of horse or cow manure put on the fields through which it runs, or polluted by a stray cow or horse. Swine, hens, birds, etc., may contaminate in unsuspected ways. Up to the present there is no conclusive evidence that colon bacilli increase for any considerable length of time anywhere except in the intestines of the higher vertebrates, and they are widely distributed in nature mainly because the fecal discharges of man and animals are a common thing on the soil. When the colon bacillus is present so as to be isolated from 1 c.c. of water in a series of tests, it is reasonable proof of pollution and the conditions should be investigated. Ten colon bacilli in 1 c.c. indicates serious pollution, and very likely a dangerous one. Winslow reports that in only two out of fifty-eight samples of presumably non-polluted waters did he get colon bacilli in

the 1 c.c. samples. Even in twenty-one stagnant pools he only found colon bacilli in five in the 1 c.c. samples.

The experience of all who have practically studied the subject is that in delicacy the colon test surpasses chemical analysis; in constancy and definiteness it also excells the quantitative bacterial test.

Interpretation of the Quantitative Analysis.—The older experimenters attempted to establish arbitrary standards by which the sanitary quality of water could be fixed automatically by the number of germs alone. This has been largely given up. Dr. Sternberg considers that a water containing less than 100 bacteria is presumably from a deep source and uncontaminated by surface drainage; that one with 500 bacteria is open to suspicion; and that one with over 1000 bacteria is presumably contaminated by sewage or surface drainage. Even this conservative opinion must be applied with caution. The source of the sample is of vital importance in the interpretation; thus, a bacterial count which would condemn a spring or well might be normal for a river. In woodland springs and lakes several hundred bacteria are frequently found. In lakes the point at which the sample is taken is of great importance, as the bacterial count varies with the distance and with the depth. The weather also is an influence, since the wind causes waves which stirs up the bottom mud. Rains greatly influence streams by flooding them with surface water. The season of the year is an important factor. The counts are highest in the winter and spring months, and lower from April to September.

The following figures illustrate this point:

Water.	Observer.	Year.	Jan.	Feb.	March.	April.	May.	June.
New York City tap-water.	Houghton	1904	890	1100	650	240	350	370
Boston "	Whipple	1892	135	211	102	52	53	86
Merrimac River "	Clark	1899	4900	5900	6300	2900	1900	3500

The spring increase is not an exception to the rule that high numbers indicate danger but an indication of its truth, for it means a melting of the snow and a flow of surface water into the streams. A number of severe epidemics of typhoid fever have been produced in this way. Although, as a rule, a series of tests are necessary to pass judgment on a water, a single test may be very important. A large increase in the number in tap-water a day after a storm points unerringly to surface pollution, and if towns exist in the water-shed, to street and sewer pollution. The Croton water frequently jumps from hundreds to thousands after such a storm.

In a typhoid epidemic at Newport, Winslow reports that a test of the water supply showed but 334 bacteria per cubic centimetre, but one from a well showed 6100. The suspicion aroused was justified by finding all the typhoid cases had gotten water from this well.

The study of the bacterial effluent from municipal water filters is the only way in which the efficiency of the filter and the accidents which occur can be determined. In Germany these regular tests are obligatory. Elaborate studies have recently been made of the exact

distribution of streams of sewage in bodies of water into which they flow, their disappearance by dilution and sedimentation, and their removal by death. Under peculiar conditions bacteria in water may increase for a time, but here the prevailing bacteria belong almost exclusively to one type.

Streptococci in Sewage.—The varieties of streptococci found most often in polluted water correspond to the streptococci described by Houston. In some water in which these are found no B. coli have been found and there is considerable doubt in such cases as to whether the streptococci imply serious pollution. The streptococci remain alive much longer than the colon bacilli, and therefore, probably, than the typhoid bacillus.

The Proteus Group.—Members of the proteus group are often found in polluted waters and but rarely in pure water. The bacillus pyocyaneus is also at times present, and has in a few cases excited infectious diarrhœa. Bacillus cloacæ is a common form of sewage bacteria.

Isolation of the Typhoid Bacillus from Water.—If it were possible to readily obtain the typhoid bacilli from water, when they were present in small numbers, its examination for that purpose would be of much greater value than it is now; but we have to remember that we can only examine at one time a few cubic centimetres of water by bacteriological methods, and that although the typhoid bacilli may be sufficiently abundant in the water to give, in the quantity that we ordinarily drink, a few bacilli, yet it must be a very lucky chance if they happen to be in the small amount which we examine. Still, further, although it is very easy to isolate typhoid bacilli from water when they are in considerable numbers, yet when they are a very minute proportion of all the bacteria present it is almost impossible not to overlook them. Many attempts have been made to devise some method by which the relative number of the typhoid and other parasitic bacteria present in water could be increased at the expense of the saprophytic bacteria. Thus to 100 c.c. of water 25 c.c. of a 4 per cent. peptone nutrient bouillon is added, and the whole put in the incubator at 37° C. for twenty-four hours. From this, plate cultures are made. In our experience this and other methods, such as collecting the bacteria left on a filter after passing several gallons through, have not enabled us to detect the typhoid bacillus where we have failed to find it by making direct plate cultures. As a matter of fact, the typhoid bacillus is rarely found, even in specimens of water where we actually know that it is or has been present because of cases of typhoid fever which have developed from drinking the water. From these facts we must consider our lack of finding the bacillus in any given case as absolutely no reason for considering the water to be free from danger. Another serious drawback to the value of the examinations for the typhoid bacillus is that they are frequently made at a time when the water is really free from contamination, though both earlier and later the bacillus was present. It is hardly worth while, therefore, except in careful experimental researches, to examine the water for the typhoid bacillus, but rather

study the location of the surrounding privies and sources of contamination. A number of observers, resting on the agglutination test, have thought they have isolated typhoid bacilli from the soil and water, but these investigators had not considered sufficiently the matter of group agglutinins, and their results are not trustworthy.

Bacteriological Examination of Air.

Saprophytic bacteria are always present in considerable numbers in the air except far out at sea or on high mountains. They are more abundant where organic matter abounds, and in dry and windy weather. Pathogenic bacteria, on the other hand, are only occasionally present in the air. The practical results obtained from the examination of air for pathogenic bacteria have been slight. We know that at times they must be in the air, but unless we purposely increase their numbers they are so few in the comparatively small amount of air which it is practicable to examine that we rarely find them. Examination of dust, however, in hospital wards and sick-rooms, in places where only air infection was possible, have revealed tubercle bacilli and other pathogenic bacteria.

The simplest method of searching for the varieties of bacteria in the air and their number in any place is to expose to the air for longer or shorter periods nutrient agar spread upon the surface of the Petri dish. After exposure the plates are put either in the incubator at 37° C. or kept at room temperature. The more careful quantitative examination is made by drawing a given quantity of air through tubes containing sterile sand, which is kept in by pieces of metal gauze. When the operation is completed the sand is poured into a tube containing melted nutrient gelatin or nutrient agar, and after thoroughly shaking, the mixture is poured into a Petri dish and the bacteria allowed to develop, either at 37° or 20° C., according as the growth of the parasitic or saprophytic varieties is desired. Instead of agar or gelatin ascitic broth or animals may be inoculated. Such examinations are occasionally made of the air of theatres, crowded streets in cities, etc. They give interesting but hardly valuable results.

Bacteriological Examination of the Soil.

The subject from its agricultural side cannot be considered here. The soil can be gathered in sterile, sharp-pointed, sheet-iron tubes. As in water, we wish to learn the number of bacteria and the important varieties of bacteria present. To estimate the number, small fractions of a gram are taken.

According to Houston uncultivated sandy soil averages 100,000 bacteria per gram, garden soil 1,500,000, and sewage-polluted 115,000,000. The most important bacteria to be sought for are bacilli of the colon group and streptococci. Both of these suggest fairly recent excremental pollution.

The period during which typhoid bacilli remain alive in soil is un-

known, since it depends on so many unknown factors and differs so in different places. The typhoid bacilli probably rarely increase in the soil and probably rarely survive a month in it. The main danger of soil bacteria is their being washed in water supplies by rains and wind.

Contamination and Purification of Drinking Waters.

Brook-water and river-water are contaminated in two ways: through chemicals, the waste products of manufacturing establishments, and through harmful bacteria by the contents of drains, sewers, etc., the latter method being by far the more dangerous.

When water, which has been soiled by waste products of manufactories only, becomes so diluted or purified that the contamination is not noticeable to the senses and shows no dangerous products on chemical analysis, it is probably safe to drink. When sewage is the contamination this rule no longer holds, and there may be no chemical impurities and no pathogenic bacteria found, and yet disease be produced. That river-water which has been fouled by sewage will, in the course of a few miles, through the dilution of additional supplies, through sedimentation, and through oxidation, become greatly purified is an indisputable fact. The increase in bacteria which occurs from contamination is also largely or entirely lost after ten to twenty miles of river flow. Nevertheless, the history of many epidemics seems to show that a badly contaminated river is never an absolutely safe water to drink, although with the lapse of each day it becomes less and less dangerous, nor will sand filter beds absolutely remove all danger. These statements are founded upon the results of numerous investigations; thus the marked disappearance of bacteria is illustrated by the following: Kummel found below the town of Rosbock 48,000 bacteria to the cubic centimetre; twenty-five kilometres farther down the stream only 200 were present—about the same number as before the sewage of Rosbock entered. On the other hand, the doubtful security of depending on a river purification is proved by such experiences as the following: In the city of Lowell, Massachusetts, an alarming epidemic followed the pollution of the Merrimac River three miles above by typhoid feces, and six weeks later an alarming epidemic attacked Lawrence, nine miles below Lowell. It was estimated that the water took ten days to pass from Lowell to Lawrence and through the reservoirs. Typhoid bacilli usually die in river-water in from three to ten days, but they may live for twenty-five days in other water; the Lawrence epidemic is easily explained. Newark-on-Trent, England, averaged seventy-five cases a year from filtered water and only ten when it was changed to deep-well supply.

Purification of Water on a Large Scale.—For detailed information on this subject the reader is referred to works on hygiene. Surface waters, if collected and held in sufficiently large lakes or reservoirs usually beome so clarified by sedimentation, except shortly after heavy rains, as to require no further treatment so far as its appearance goes.

The collection of water in large reservoirs allows not only the living and dead matter to subside, but allows time also for the pathogenic germs to perish through light and antagonistic bacteria and other deleterious influences, sand or mechanical coagulant. Filtration of water exerts a very marked purification, taking out 99.8 per cent. of the organisms in those best constructed and at least 95 per cent. in those commonly used in cities. The construction of filters is too large a subject to enter on minutely here; sand filters consist, as a rule, of several layers, beginning with fine sand, and then smaller and larger gravel, and finally rough stones. A certain time elapses before the best results are obtained; this seems to wait for the formation of a film of organic material on the sand, which is full of nitrifying bacteria. Even the best filters only greatly diminish the dangers of polluted water. Spring-water and well-water are, in fact, filtered waters.

Water which is subject to serious pollution must be submitted to a preliminary purification before it can be considered a suitable source for a drinking-water supply. The means employed for its purification depend to a large extent upon the character of the water and the nature of the pollution. Filtration through slow sand filters, three to five feet in depth, removes 98 to 99.5 per cent. of the bacteria and organic matter; so that effluents from the best constructed sand filtration beds constitute safe and reliable drinking waters. Five hundred thousand to one or two million gallons, depending somewhat upon the extent of pollution and the fineness of the sand, can be filtered daily per acre. Only the surface of the sand filter becomes in any way clogged and as thin a layer as can be scraped off is removed one or more times a month. This surface sand is washed with clean water and several scrapings replaced at one time. Sand filtration beds are very widely used abroad and are coming into extensive use in this country. The filter beds at Lawrence, Mass., have been used over ten years with marked success, rendering the highly polluted Merrimac River a safe drinking-water; the filter beds there are scraped about thirteen times a year.

Mechanical filtration plants find considerable favor where clarification as well as bacterial purification is desired. A coagulant such as sulphate of aluminum is employed and forms in the water a flocculent precipitate which carries down with it all suspended matter; 50,000,000 or more gallons of water can be filtered on an acre daily, but the filters must be washed once or twice daily by reversing the flow and cleansing the clogged filter with a stream of the purified water. The bad clogging is due to the fact that the process is a purely mechanical one, and not comparable in any way with the vital processes carried on in the sand filter by the nitrifying bacteria.

Other methods are coming into use, such as the passage of ozone, and have proved successful. Such processes should be under the direct supervision of expert sanitary engineers and bacteriologists.

Domestic Purification.—Water which requires private filtering should not be supplied for drinking purposes. Unhappily, however, it often

is. Filters may be divided, roughly, into those for low and high pressure. The former are directly connected with the water main, while the others simply have the slight pressure of the column of water standing in the filter. Many high-pressure filters contain animal charcoal, silicated carbon, etc., either in a pressed condition or in one porous mass. These filters remove much of the deleterious matter from the suspected waters, but the majority cannot be depended upon to remove all bacteria. Even those which are equipped for self-cleansing become in a little while foul, and, if not cleaned, unfit for use. The best of the class are of porous stone, such as the Berkefeld and Pasteur filters. These yield a water, if too great pressure is not used, almost absolutely free from bacteria, and if they are frequently cleansed they are reliable. A large Berkefeld filter will allow sixty gallons of water to pass per hour. The Pasteur filter is more compact and slower. From the best Pasteur filters sterile water may be passed for two to three weeks; from the Berkefeld usually only a few days. A simple typical low-pressure filter is that of Bailey Denton. The upper compartment contains the filtering material, which may be sand or charcoal, and is fed from a cistern or hydrant. After a certain quantity of water has passed in, the supply is automatically cut off until the whole amount has filtered. A filter easily made is the following: Take a large-sized earthenware pot and plug the hole in the bottom with a cork, through which pass a short glass tube. Upon the bottom place an inch of small pieces of broken flower-pot; upon this a couple of inches of well-washed small gravel, and upon this six to twelve inches of well-washed, fine, sharp sand. Cover the sand with a piece of filter paper and hold this down with a few small stones. Mount the pot on a tripod, and it is ready for use. The paper prevents the sand being disturbed when water is added, and as it also holds most of the sediment, this can be readily removed. Every few months the sand can be washed and replaced. Animal charcoal is not a good substance for permanent filters, as bacteria grow well in it. Whenever water is suspected, and there is any doubt as to the filters, it should be boiled for ten minutes; this will destroy all bacteria. This precaution should always be taken in the presence of typhoid fever and cholera epidemics.

The Disposal of Sewage.

The disposal of sewage is becoming a vital question with all towns and cities which are not situated near salt-water outlets, since the present tendency in legislation is to compel such towns to dispose of their waste so that it shall not be a menace to drinking-water streams, destructive to fisheries, or a nuisance to harbors.

Methods of sewage purification depends upon the character of the sewage and the kind of effluent desired.

Two hundred thousand gallons of crude sewage may be filtered upon an acre of land daily and an effluent obtained which will compare favorably in every way known to the chemist and bacteriologists with the best mountain springs. This is, however, a slow process and

it is rare that such a pure effluent is required. Similar results may be obtained by utilizing the septic tank method, running the sewage from the septic tank to contact beds and thence to sand filter beds; where because of the partial "self-purification of the sewage" in the septic tank and contact beds 2,500,000 gallons of sewage can be filtered daily on an acre of surface. In this process less land is required and both these effluents can be safely turned into drinking-water streams.

If, however, a merely non-putrescible effluent is required, one which, though high bacterially, will not be offensive in any way, or subject to further decomposition, it may be obtained by passing crude sewage to septic tanks, thence to double contact beds, the resulting effluent having merely an earthy, humus-like odor and being non-putrescible.

Where acid wastes, tannery wastes, dyestuffs, etc., from various factories enter into sewage, its disposal becomes a more complicated problem and chemical precipitation by the use of lime or other chemicals is generally employed for such sewage purification, which at best is only partial and is sometimes supplemented by sand filtration.

CHAPTER XXXVI.

THE BACTERIOLOGY OF MILK IN ITS RELATION TO DISEASE.

FROM the standpoint of the dairy and cheese producer a study of the different varieties of bacteria of the air and dust are of importance. Some yield products which give the butter, cheese, milk, or cream a bad taste or odor. We can only consider here the bacteriology of milk so far as it is related to health and disease.

The bacteria in milk can be divided into two great groups—those which get into the milk after it leaves the udder and those which come from the cow. The first group comprises bacteria from dust, etc.

The extraneous bacteria are of importance because they produce changes in the chemical composition of the milk when they have developed in great numbers. The number of bacteria in any sample of milk depends on three factors: the number deposited in the milk from the cow's udder, from the air, and utensils; the time during which they have developed, and the temperature at which the milk has stood. The last is perhaps the most important. The attempt was made during the past three years to connect illness in children with the varieties of bacteria in milk. As a matter of fact no such connection was made out. The number of varieties was found to be so great that if any special type of disease had developed in those getting milk it would have been an extremely difficult thing to have made out such a connection.[1] This in spite of the fact that the varieties isolated represent only the species present in greatest number in the milk examined, for in no case was more than 0.01 c.c. of a milk, and in most highly contaminated milks only 0.001 c.c., used in making a plate, and varieties which occurred in too small numbers to be present in this quantity would necessarily be missed. For the purposes of this book it is not considered desirable to burden the reader with the enumeration of the varieties of bacteria found in the different samples of milk and their characteristics. Only a brief summary of the results will be given.

[1] The bacteria were isolated from the milk through plating in a 2 per cent. lactose-litmus nutrient gelatin or agar, and later grown upon the usual identification media. The pathogenic properties of the different bacteria were tested by intraperitoneal and subcutaneous inoculation in guinea-pigs with 2 c.c. of a forty-eight-hour broth culture, and by feeding young kittens for several days with 3 to 6 c.c. daily of a twenty-four-hour broth culture by means of a medicine dropper.

With the characteristics of the bacteria thus determined, they were then separated into classes following as nearly as possible the lines suggested in Chester's Manual of Determinative Bacteriology. Further attempt was then made to identify as many as possible of the varieties with those previously described, using the descriptions of Chester and Migula. With a great many this proved unsatisfactory or impossible because of the incomplete descriptions in literature, or the lack of all description.

From the milks altogether, 239 varieties of bacteria were isolated and studied. These 239 varieties, having some cultural or other differences, were divided into the 31 classes, each class containing from 1 to 39 more or less closely related organisms.

As to the sources of bacteria found in milk, we made sufficient experiments to satisfy us that they came chiefly from outside the udder and milk-ducts.

Bacteria were isolated from various materials which under certain conditions might be sources of contamination for the milk, and the cultures compared with those taken from milk. Thus there were obtained from 20 specimens of hay and grass, 31 varieties of bacteria; from 15 specimens of feces, manure, and intestinal contents, 28 varieties; from 10 specimens of feed, 17 varieties. Of these 76 varieties there were 26 which resembled closely those from milk—viz., 11 from grass or hay; 26 from manure; 5 from feed.

During the investigation a number of the varieties isolated from milk were shown to be identical with types commonly found in water.

From the few facts quoted above and from many other observations made during the course of the work, it would seem that the term "milk bacteria" assumes a condition which does not exist in fact. The expression would seem to indicate that a few varieties, especially those derived in some way from the cow, are commonly found in milk, which forms having entered the milk while still in the udder, or after its withdrawal, are so well fitted to develop in milk that they overgrow all other varieties.

As a matter of fact, it was found that milk taken from a number of cows, in which almost no outside contamination had occurred, and plated immediately, contained, as a rule, very few bacteria, and these were streptococci, staphylococci, and other varieties of bacteria not often found in milk sold in New York City; the temperature at which milk is kept being less suitable for them than for the bacteria which fall into the milk from dust, manure, etc. A number of specimens of fairly fresh market milk averaging 200,000 bacteria per cubic centimetre were examined immediately, and again after twelve to twenty-four hours. In almost every test the three or four predominant varieties of the fresher milk remained as the predominant varieties after the period mentioned.

The above experiments seem to show that organisms which have gained a good percentage in the ordinary commercial milk at time of sale will be likely to hold the same relative place for as long a period as milk is usually kept. After the bacteria pass the ten or twenty million mark a change occurs, since the increasing acidity inhibits the growth of some forms before it does that of others. Thus some varieties of the lactic acid bacteria can increase until the acidity is twice as great as that which inhibits the growth of streptococci. Before milk reaches the curdling point, the bacteria have usually reached over a billion to each cubic centimetre. For the most part specimens of milk from different localities showed a difference in the character of the bacteria present, in the same way that the bacteria from hay, feed, etc., varied. Even the intestinal contents of cows, the bacteriology of which might be expected

to show common characteristics, contained, besides the predominating colon types, other organisms which differed widely in different species and in different localities. Cleanliness in handling the milk and the temperature at which it had been kept were also found to have a marked influence on the predominant varieties of bacteria present.

Pathogenic Properties of the Bacteria Isolated.—Intraperitoneal injection of 2 c.c. of broth or milk cultures of about 40 per cent. of the varieties tested caused death. Cultures of most of the remainder produced no apparent deleterious effects even when injected in larger amounts. The filtrates of broth cultures of a number of varieties were tested, but only one was obtained in which poisonous products were abundantly present. Death in guinea-pigs weighing 300 grams followed within fifteen minutes after an injection of 2 c.c.; 1 c.c. had little effect.

As bacteria in milk are swallowed and not injected under the skin, it seemed wise to test the effect of feeding them to every young animals. We therefore fed forty-eight-hour cultures of 139 varieties of bacteria to kittens of two to ten days of age by means of a glass tube. The kittens received 5 to 10 c.c. daily for from three to seven days. Only one culture produced illness or death. A full report on the identification of the varieties of bacteria met with in this investigation can be found in an article by Dr. Letchworth Smith in the 1902 *Annual Report of the Department of Health.*

After three years of effort to discover some relation between special varieties of bacteria found in milk and the health of children the conclusion has been reached that neither through animal tests nor the isolation from the milk of sick infants have we been able to establish such a relation. Pasteurized or "sterilized" milk is rarely kept in New York longer than thirty-six hours, so that varieties of bacteria which after long standing develop in such milk did not enter into our problem. The harmlessness of cultures given to healthy young kittens does not of course prove that they would be equally harmless in infants. Even if harmless in robust infants, they might be injurious when summer heat and previous disease had lowered the resistance and the digestive power of the subjects. In a recent investigation by Dr. D. H. Bergey some connection between diarrhœa and pus and streptococci were found.

The results of this investigation appear to warrant the following conclusions:

1. The occurrence of pus in cows' milk is probably always associated with the presence in the udder of some inflammatory reaction brought about by the presence of some of the ordinary pyogenic bacteria, especially of streptococci.

2. When a cow's udder has once become infected with the pyogenic bacteria the disease tends to persist for a long time, probably extending over several periods of lactation.

3. Lactation has no causative influence *per se* upon the cellular and bacterial content of cows' milk, though it probably tends toward the aggravation of the disease when the udder is once infected.

4. The so-called "gelbe galt," or contagious mammitis of European writers, appears to be merely a severe form of mammitis due to a variety of streptococcus which, on account of its chromogenic properties, gives to the milk its peculiar golden-yellow color.

Our failure to discover definite pathogenic bacteria in milk, as well as the numerous varieties of bacteria met with, have forced us to rely on the clinical observation of infants to note what difference, if any, occurred in those fed on raw and Pasteurized milk from the same source, and upon different milks of unknown origin varying in the number of bacteria contained. These observations were combined with those upon other factors which influenced the health of the infants.

Heated Milk vs. Raw Milk for Infants.—During each of the summers of 1902, 1903, and 1904 a special lot of milk was modified at one of the Straus depots for a group of fifty infants, all of whom were under nine months of age, and distributed daily in the usual way. To one-half the infants the milk was given raw; to the other half Pasteurized.

The modified milk was made from a fairly pure milk mixed with ordinary cream. The bacteria contained in the milk numbered on the average 45,000 per cubic centimetre, in the cream 30,000,000. The modified raw milk taken from the bottles in the morning averaged 1,200,000 bacteria per cubic centimetre, or considerably less than the ordinary grocery milk; the Pasteurized, about 1000; taken in the late afternoon of the same day they had respectively about 20,000,000 and 50,000.

Twenty-one predominant varieties of bacteria were isolated from six specimens of this milk collected on different days. The varieties represented the types of bacteria frequently found in milk. The infants were selected during the first week in June, and at first all were placed on Pasteurized milk. The fifty infants which had been selected were now separated into two groups as nearly alike as possible. On the15th of June the milk was distributed without heating to one-half the infants, the other half receiving as before the heated milk. In this way the infants in the two groups received milk of identically the same quality, except for the changes produced by heating to 165° F. for thirty minutes. The infants were observed carefully for three months and medical advice was given when necessary. When severe diarrhœa occurred barley-water was substituted for milk.

The first season's trial gave the following results: Within one week 20 out of the 27 infants put on the raw milk suffered from moderate or severe diarrhœa; while during the same time only 5 cases of moderate and none of severe diarrhœa occurred in those taking Pasteurized milk. Within a month 8 of the 27 had to be changed from raw back to heated milk, because of their continued illness; 7, or 25 per cent., did well all summer on raw milk. On the other hand, of those receiving the Pasteurized milk, 75 per cent. remained well, or nearly so, all summer, while 25 per cent. had one or more attacks of severe diarrhœa. There were no deaths in either group of cases.

During the second summer a similar test was made with 45 infants.

Twenty-four were put on raw modified milk; 13 of these had serious diarrhœa, in 5 of whom it was so severe that they were put back upon heated milk; 10 took raw milk all summer without bad effects; 2 died, 1 from gross neglect on the part of the mother, the other from diarrhœa. Of the 21 on Pasteurized milk, 5 had severe attacks of diarrhœa, but all were kept on this milk except for short periods, when all food was omitted; 16 did well throughout the summer. One infant, markedly rachitic, died. The third summer's results have not been tabulated, but were similar to those of the first two tests.

The outcome of these observations during the first two summers are summarized in the following table:

Kind of milk.	Number of infants	Remained well for entire summer.	Number having severe or moderate diarrhœa.	Average number days off milk during summer.	Average weekly gain in weight.	Aver'ge number of days diarrhœa.	Deaths.
Pasteurized milk, 1000 to 50,000 bacteria per c.c.	41	31	10	3	4.0 oz.	3.9	1
Raw milk, 1,200,000 to 20,000,000 bacteria per c.c.	51[1]	17	33	5.5	3.5 "	11.5	2

Although the number of cases was not large, the results, almost identical during the three summers, indicate that even a fairly pure milk when given raw, in hot weather, causes illness in a much larger percentage of cases than the same milk given after Pasteurization. A considerable percentage of infants, however, do apparently quite as well on raw as on Pasteurized wilk.

Heated Milk vs. Raw Milk for Older Children.—The children over three years of age who received unheated milk, containing at different times from 145,000 to 350,000,000 bacteria per cubic centimetre, showed almost no gastrointestinal disturbance. The conditions at three institutions will serve as examples.

In the first of these an average grade of raw milk was used which, during the summer contained from 2,000,000 to 30,000,000 bacteria per cubic centimetre. This milk was stored in an ice-box until required. It was taken by the children unheated and yet no case of diarrhœa of sufficient gravity to send for a physician occurred during the entire summer. This institution was an orphan asylum containing 650 children from three to fourteen years of age—viz., three to five years, 98; five to eight years, 162; eight to fourteen years, 390.

A second institution used an unheated but very pure milk which was obtained from its own farm. This milk averaged 50,000 bacteria per cubic centimetre. The inmates were 70 children of ages ranging from three to fourteen years. In this institution not a single case of diarrhœal disease of any importance occurred during the summer.

[1] Thirteen of the fifty-one infants on raw milk were transferred before the end of the trial to Pasteurized milk because of serious illness. If these infants had been left on raw milk it is believed by the writers that the comparative results would have been even more unfavorable to raw milk.

In a third institution an average grade of milk was used which was heated. This milk before heating contained 2,000,000 to 20,000,000 bacteria per cubic centimetre. The institution was an infant asylum in which there were 126 children between the ages of two and five years. There were no cases of diarrhœa during the summer.

These clinical observations taken in connection with the bacteriological examination at the laboratory show that although the milk may come from healthy cattle and clean farms, and be kept at a temperature not exceeding 60° F., a very great increase in the number of bacteria may occur. Furthermore, this may occur without the accumulation in the milk of sufficient poisonous products or living bacteria to cause appreciable injury in children over three years of age, even when such milk is consumed in considerable amount and for a period extending over several months. Milk kept at temperatures somewhat above 60° F. was not met with in our investigations, but the histories of epidemics of ptomain poisoning teach that such milk may be very poisonous. It is also to be remembered that milk abounding in bacteria on account of its being carelessly handled is also always liable to contain pathogenic organisms derived from human or animal sources.

Results with Very Impure Milk Heated vs. Those with Pure or Average Milk Heated.—During the summer of 1901 we were able to observe a number of babies fed on milk grossly contaminated by bacteria. In 1902, systematic supervision of all stores selling milk was instituted by the Health Department, so that the very worst milk was not offered for sale that summer.

The observations upon the impure milk of 1901 are of sufficient importance to be given in detail, although already mentioned in the report of the observations upon infants of both summers which were fed on "store milk." A group of over 150 infants was so divided that 20 per cent. were allowed to remain on the cheapest store milk which they were taking at the time. To about the same number was given a pure bottled milk. A third group was fed on the same quality of milk as the second, but sterilized and modified at the Good Samaritan Dispensary. A fourth group received milk from an ordinary dairy farm. This milk was sent to a store in cans and called for by the people. A few infants fed on breast and condensed milk were observed for control.

In estimating the significance of the observations recorded in the tables, one should bear in mind that not only do different infants possess different degrees of resistance to disease, but that, try as hard as the physicians could, it was impossible to divide the infants into groups which secured equal care, and were subjected to exactly the same conditions. It was necessary to have the different groups in somewhat different parts of the city. It thus happened that the infants on the cheap store milk received less home care than the average, and that those on the pure bottled milk lived in the coolest portion of the city. Certain results were, however, so striking that their interpretation is fairly clear. It is to be noted that the number of infants included in each group is small.

TABLE SHOWING THE RESULTS OF FEEDING DURING JULY AND AUGUST, 1901, IN TENEMENT-HOUSES, OF 112 BOTTLE-FED INFANTS UNDER 1 YEAR OF AGE, AND OF 47 BOTTLE-FED INFANTS BETWEEN 1 AND 2 YEARS OF AGE WITH MILK FROM DIFFERENT SOURCES, AND THE NUMBER OF BACTERIA PRESENT IN THE MILK.

Character of the milk.	Infants under one year.					Infants over one year.				
	Number of infants.	Average weekly gain.	Diarrhœa. Mild.	Diarrhœa. Severe.	Deaths.	Number of infants.	Average weekly gain.	Diarrhœa. Mild.	Diarrhœa. Severe.	Deaths.
1. Pure milk boiled and modified at dispensary or stations; given out in small bottles. Milk before boiling averaged 20,000 bacteria per c.c.; after boiling 2 per c.c.	41	3 oz.	10	8	1[1]					
2. Pure milk, 24 hours old, sent in quart bottles to tenements, heated and modified at home, 20,000 to 200,000 bacteria per c.c. when delivered.	28	4⅙ "	8	5	0	24	4⅓ oz.	8	2	0
3. Ordinary milk, 36 hours old, from a selected group of farms, kept cool in cans during transport; 1,000,000 to 25,000,000 bacteria per c.c., heated and modified at home before using.	18	4 "	6	6	1[2]	12	4 "	1	2	0
4. Cheap milk, 36 to 60 hours old, from various small stores, derived from various farms, some fairly clean, some very dirty; 400,000 to 175,000,000 bacteria per c.c.	21	¼ "	4	13	4[3]	7	⅓ "	1	3	0
5. Condensed milk of different brands. Made up with hot water. As given, contained bacteria from 5000 to 200,000 per c.c.	9	¼ "	5	2	3	4	3⅞ "	1	3	0
6. Breast milk	16	2¼ "	5	2	0					

There is nothing in the observations to show that fairly fresh milk from healthy cows, living under good hygienic conditions and containing, on some days, when delivered, as many as 200,000 bacteria per cubic centimetre, had any bacteria or any products due to bacteria that remained deleterious after the milk was heated to near the boiling point.

On the other hand, it is possible that certain varieties of bacteria may, under conditions that are unsanitary, find entrance to milk and survive moderate heat or may develop poisonous products resistant to heat in sufficient amount to be harmful, even when they have accumulated to less than 200,000 per cubic centimetre.

Turning now to the results of feeding with milk which has been heated and which before sterilization contained from 1,000,000 to

[1] This infant died from enteritis and toxæmia.
[2] This infant died of pneumonia. There had been no severe intestinal disorder noted.
[3] One of the four had pertussis, the remaining three died from uncomplicated enteritis.

25,000,000 bacteria per cubic centimetre, averaging about 15,000,000, though obtained from healthy cows living under fairly decent conditions and although the milk was kept moderately cool in transit, we find a distinct increase in the amount of diarrhœal diseases. Though it is probable that the excessive amount of diarrhœa in this group of children was due to bacterial changes which were not neutralized by heat or to living bacteria which were not killed, yet it is only fair to consider that the difference was not very great and that the infants of this group were under surroundings not quite as good as those on the purer milk.

Finally, we come in this comparison to the infants who received the cheap store milk Pasteurized. This milk had frequently to be returned because it curdled when boiled, and contained, according to the weather, from 4,000,000 to 200,000,000 bacteria per cubic centimetre. In these infants the worst results were seen. This is shown not only by the death rate, but by the amount and the severity of the diarrhœal diseases, and the general appearance of the children as noted by the physicians. Although the average number of bacteria in the milk received by this group is higher than that received by the previous group, the difference in results between this group and the previous one can hardly be explained by the difference in the number of bacteria. The varieties of bacteria met with in this milk were more numerous than in the better milk, but we were unable to prove that they were more dangerous. Probably the higher temperature at which the milk was kept in transit and the longer interval between milking and its use allowed more toxic bacterial products to accumulate.

Summary.—The observations here recorded were made upon the groups of infants for periods of about three months only, and the conclusions[1] drawn relate especially to the more immediate effects of the milk:

1. During cool weather neither the mortality nor the health of the infants observed in the investigation was appreciably affected by the kind of milk or by the number of bacteria which it contained. The different grades of milk varied much less in the amount of bacterial contamination in winter than in summer, the store milk averaging only about 750,000 bacteria per cubic centimetre.

2. During hot weather when the resistance of the children was lowered, the kind of milk taken influenced both the amount of illness and the mortality; those who took condensed milk and cheap store milk did the worst, and those who received breast milk, pure bottled milk, and modified milk did the best. The effect of bacterial contamination was very marked when the milk was taken without previous heating; but, unless the contamination was very excessive, only slight when heating was employed shortly before feeding.

3. The number of bacteria which may accumulate before milk becomes noticeably harmful to the average infant in summer differs with the nature of the bacteria present, the age of the milk, and the

[1] These conclusions were drawn up by the writer in association with Dr. L. E. Holt, after a joint study of the results obtained in the studies above recorded.

temperature at which it has been kept. When milk is taken raw, the fewer the bacteria present the better are the results. Of the usual varieties, over 1,000,000 bacteria per cubic centimetre are certainly deleterious to the average infant. However, many infants take such milk without apparently harmful results. Heat above 170° F. (77° C.) not only destroys most of the bacteria present, but, apparently, some of their poisonous products. No harm from the bacteria previously existing in recently heated milk was noticed in these observations unless they had amounted to many millions, but in such numbers they were decidedly deleterious.

4. When milk of average quality was fed sterilized and raw, those infants who received milk previously heated did, on the average, much better in warm weather than those who received it raw. The difference was so quickly manifest and so marked that there could be no mistaking the meaning of the results. The bacterial content of the milk used in the test was somewhat less than in the average milk of the city.

5. No special varieties of bacteria were found in unheated milk which seemed to have any special importance in relation to the summer diarrhœas of children. The number of varieties was very great, and the kinds of bacteria differed according to the locality from which the milk came. None of the 139 varieties selected as most distinct among those obtained injured very young kittens when fed in pure cultures. A few cases of acute indigestion were seen immediately following the use of Pasteurized milk more than thirty-six hours old. Samples of such milk were found to contain more than 100,000,000 bacteria per cubic centimetre, mostly spore-bearing varieties. The deleterious effects, though striking, were neither serious nor lasting.

At the present time there is in New York City no general sale from stores of "Pasteurized" or "sterilized" milk; so that here it is very rare for such milk to be used thirty-six hours after heating.

6. After the first twelve months of life, infants are less and less affected by the bacteria in milk derived from healthy cattle. According to these observations, when the milk had been kept cool the bacteria did not appear to injure the children over three years of age, at any season of the year, unless in very great excess.

7. Since a large part of the tenement population must purchase its milk from small dealers, at a low price, everything possible should be done by health boards to improve the character of the general milk supply of cities by enforcing proper legal restrictions regarding its transportation, delivery, and sale. Sufficient improvements in this respect are entirely feasible in every large city to secure to all a milk which will be wholesome after heating. The general practice of heating milk, which has now become a custom among the tenement population of New York, is undoubtedly a large factor in the lessened infant mortality during the hot months.

8. Of the methods of feeding now in vogue that by milk from central distributing stations unquestionably possesses the most advantages,

in that it secures some constant oversight of the child, and, since it furnishes the food in such a form that it leaves the mother least to do, it gives her the smallest opportunity of going wrong. This method of feeding is one which deserves to be much more extensively employed, and might, in the absence of private philanthropy, wisely be undertaken by municipalities and continued for the four months from May 15th to September 15th.

9. The use, for infants, of milk delivered in sealed bottles, should be encouraged whenever this is possible, and its advantages duly explained. Only the purest milk should be taken raw, especially in summer.

10. Since what is needed most is intelligent care, all possible means should be employed to educate mothers and those caring for infants in proper methods. This, it is believed, can most effectively be done by the visits of properly qualified trained nurses or women physicians to the homes, supplemented by the use of printed directions.

11. Bad surroundings, though contributing to bad results in feeding, are not the chief factors. It is not, therefore, merely by better housing of the poor in large cities that we will see a great reduction in infant mortality.

12. While it is true that even in tenements the results with the best bottle feeding are nearly as good as average breast feeding, it is also true that most of the bottle feeding is at present very badly done; so that, as a rule, the immense superiority of breast feeding obtains. This should, therefore, be encouraged by every means, and not discontinued without good and sufficient reasons. The time and money required for artificial feeding, if expended by the tenement mother to secure better food and more rest for herself, would often enable her to continue nursing with advantage to her child.

13. The injurious effects of table food to infants under a year old, and of fruits to all infants and young children in cities, in hot weather, should be much more generally appreciated.

Influence of Temperature upon the Multiplication of Bacteria in Milk.—Few, even of the well informed, appreciate how great a difference a few degrees of temperature will make in the rate of bacterial multiplication. Milk rapidly and sufficiently cooled keeps almost unaltered for thirty-six hours, while milk insufficiently cooled deteriorates rapidly.

The majority of the bacteria met with in milk grow best at temperatures above 70° F., but they also multiply slowly even at 40° F.; thus of 60 species isolated by us, 42 developed good growths at the end of seven days at 39° F. Our observations have shown that the bacteria slowly increase in numbers after the germicidal properties of the milk have disappeared, and the germs have become accustomed to the low temperature. In fact, milk cannot be permanently preserved unaltered unless kept at 32° F. or less. The degree of cooling to which ordinary supplies of milk are subjected differs greatly in various localities. Some farmers chill their milk rapidly, by means of pipe-coils over which the milk flows; others use deep wooden tanks filled with water

into which the cans of milk are placed soon after milking. In winter these methods are very satisfactory, for the water runs into the pipes or tanks at about 38° F. In warmer weather they are unsatisfactory, unless ice is used, as the natural temperature of the water may be as high as 55° F. A considerable quantity of milk is not cooled at all at the farms. It is sent to the creamery or railroad after two to six hours, and is then more or less cooled. These few hours in summer, when the milk is left almost at blood heat, allow an enormous development of bacteria to take place, as is shown in the table below.

TABLE I.—Showing the development of bacteria in two samples of milk maintained at different temperatures for twenty-four, forty-eight, and ninety-six hours, respectively. The first sample of milk was obtained under the best conditions possible, the secoud in the usual way. When received, specimen No. 1 contained 3000 bacteria per c.c., specimen No. 2, 30,000 per c.c.

Temperature. Fahrenheit.	Time which elapsed before making test. 24 hrs.	48 hrs.	96 hrs.	168 hrs.
32°	2400	2100	1850	1400
	30,000[1]	**27,000**	**24,000**	**19,000**
39°	2500	3600	218,000	4,209,000
	38,000	**56,000**	**4,300,000**	**38,000,000**
42°	2600	3600	500,000	11,200,000
	43,000	**210,000**	**5,760,000**	**120,000,000**
46°	3100	12,000	1,480,000	80,000,000
	42,000	**360,000**	**12,200,000**	**300,000,000**
50°	11,600	540,000	300,000,000	
	89,000	**1,940,000**	**1,200,000,000**	
55°	18,800	3,400,000		
	187,000	**38,000,000**		
60°	180,000	28,000,000		
	900,000	**168,000,000**		
68°	450,000			
	4,000,000			
86°	1,400,000,000			
	14,000,000,000			

Observations on Bacterial Multiplication in Milk at 90° F., a Temperature Common in New York in Hot Summer Weather.

TABLE II.—*Number of Bacteria per 1 c.c.*

Milk I. Fresh and of good quality.		Milk II. Fair quality from store.	Milk III. Bad quality from store.
Original number	5200	92,000	2,600,000
After two hours	8400	184,000	4,220,000
" four "	12,400	470,000	19,000,000
" six "	68,500	1,260,000	39,000,000
" eight "	654,000	6,800,000	124,000,000

A sample of milk No. I. removed after six hours and cooled to 50° F. contained 145,000,000 at the end of twenty-four hours. Some of this milk, kept cool from the beginning, contained but 12,800 bacteria per c.c. at the end of twenty-four hours.

[1] The figures referring to tests of the second sample are printed in heavy-face type.

1. The number of bacteria present at the time of milking and twenty-four, forty-eight, and seventy-two hours afterward in milk obtained and kept under correct conditions.

No preservatives were present in any of the following specimens:

Number of Bacteria in Pure Commercial Milk.

TABLE III.—Milk obtained where every reasonable means was taken to ensure cleanliness. The long hairs on the udder were clipped; the cows roughly cleaned and placed in clean barns before milking; the udders were wiped off just previous to milking; the hands of the men were washed and dried; the pails used had small (six-inch) openings, and were thoroughly cleaned and sterilized by steam before use. Milk cooled within one hour after milking to 45° F., and subsequently kept at that temperature. The first six specimens were obtained from individual cows; the last six from mixed milk as it flowed at different times from the cooler. Temperature of barns 55° F.

Number of Bacteria in 1 c.c. of Milk.[1]

	5 hrs after milking.	After 24 hrs.	After 48 hrs.	After 72 hrs.
	From six individual cows.			
	500	700	12,500	Not counted.
	700	700	29,400	" "
	19,900	5200	24,200	" "
	400	200	8,600	" "
	900	1600	12,700	" "
	13,600	3200	19,500	" "
Average	6000	1933	17,816	
	From mixed milk of entire herd.			
	6900	12,000	19,800	494,000
	6100	2,200	20,200	550,000
	4100	700	7,900	361,000
	1200	400	7,100	355,000
	6000	900	9,800	445,000
	1700	400	8,700	389,000
Average	4333	2766	10,583	329,000

Twenty-five samples taken separately from individual cows on another day and tested immediately averaged 4550 bacteria per c.c. and 4500 after twenty-four hours. These twenty-five specimens were kept at between 45° and 50° F.

2. Milk taken during winter in well-ventilated, fairly clean, but dusty barns. Visible dirt was cleaned off the hair about the udder before milking. Milkers' hands were wiped off, but not washed. Milk pails and cans were clean, but the straining cloths dusty. Milk cooled within two hours after milking to 45° F.

[1] Number of bacteria obtained from development of colonies in nutrient agar in Petri plates. The nutrient medium contained 2 per cent. peptone and 1.2 per cent. agar, and was faintly alkaline to litmus. One set of plates were usually left four days at about 20° C., and one set twenty-four hours at 37° C., and then twenty-four hours at 20° C. From 5 to 30 per cent. more colonies developed as a rule in the plates kept at room temperature than in those kept for twenty-four hours at 37° C. The milk was diluted as desired with 100 or 10,000 parts of sterile water, and 1 c.c. of the diluted milk was added to 8 c.c. of melted nutrient agar. Plates containing over 1000 colonies were found to be inaccurate, in that they gave too low totals. Apparently a considerable number of bacteria failed to develop colonies when too many were added to the nutrient agar. Nutrient gelatin was found to be more troublesome and not to yield more accurate results than nutrient agar.

TABLE IV.—*Number of Bacteria in 1 c.c. of Milk.*

	At time of milking.	After 24 hrs.	After 48 hrs.
	12,000	14,000	57,000
	13,000	20,000	65,000
	21,500	31,000	106,000
Average	15,500	21,666	76,000

Number in City Milk.

3. The condition of the average city milk is very different, and is shown in the following tables.

The twenty samples were taken late in March by Inspectors of the Department of Health of New York City from cans of milk immediately upon their arrival in the city.

The temperature of the atmosphere averaged 50° F. during the previous twenty-four hours. The temperature of the milk when taken from the cans averaged 45° F. Much of this milk had been carried over two hundred miles. From the time of its removal from the cans, which was about 2 A.M., until its dilution in nutrient agar, at 10 A.M., the milk was kept at about 45° F.

TABLE V.

From New York and Hudson River Railroad.		*From Harlem Railroad.*	
No. of sample.	No. of bacteria in 1 c.c.	No. of sample.	No. of bacteria in 1 c.c.
50	35,200,000	48	6,200,000
51	13,000,000	49	2,200,000
52	2,500,000	50	15,000,000
53	1,400,000	51	70,000
54	200,000	52	80,000
55	600,000	53	320,000

While the above figures indicate that much of the milk sold is fair, even in summer, they show an appalling condition for most of that sold to the poorer classes—those who not only comprise the larger part of the population, but who are also compelled to keep their children in town during the hot weather.

It must be kept in mind that milk averaging 3,000,000 bacteria per cubic centimetre will, when kept at the temperature common in the homes of the poor, soon contain very largely increased numbers and show its dangerous condition by turning sour and curdling.

Cleanliness Used in Obtaining Milk, and its Influence.—The present conditions under which much of the milk is obtained are not pleasant to consider. In winter, and to a less extent at other seasons of the year, the cows in many stables stand or lie down in stalls in the rear portion of which there is from one to four inches of manure and urine. When milked the hands of the milkers are not cleansed, nor are the under portions of the cows, only visible masses of manure adhering to the hair about the udder being removed. Some milkers even moisten their hands with milk, to lessen friction, and thus wash off the dirt of their hands and of the cow's teats into the milk in the pails. Some may regard it as an unnecessary refinement to ask that farmers should roughly clean the floors of their stalls once each day, that no sweeping should be done just before milking, and that the udders should be

wiped with a clean damp cloth and the milkers should thoroughly wash and wipe their hands before commencing milking. The pails and cans should not only be carefully cleansed, but afterward scalded out with boiling water. The washing of the hands would lessen the number of ordinary filth bacteria in the milk, and diminish risk of transmitting to milk human infectious diseases like scarlet fever, diphtheria, and enteric fever, by the direct washing off of the disease germs from infected hands. It would also inculcate general ideas of the necessity of cleanliness and of the danger of transmitting disease through milk. The value of cleanliness in limiting the number of bacteria is demonstrated by the figures contained in the tables.

Summary and Conclusions.—Because of its location and its hairy covering the cow's udder is always more or less soiled with dirt and manure unless cleaned. On account of the position of the pail and the access of dust-laden air it is impossible to obtain milk by the usual methods without mingling with it a considerable number of bacteria. With suitable cleanliness, however, the number is far less than when filthy methods are used, there being no reason why fresh milk should contain in each cubic centimetre on the average, more than 12,000 bacteria per c.c. in warm weather and 5000 in cold weather. Such milk, if quickly cooled to 46° F., and kept at that temperature, will at the end of thirty-six hours contain on the average less than 50,000 bacteria per cubic centimetre, and if cooled to 40° F. will average less than its original number.

With only moderate cleanliness such as can be employed by any farmer without adding appreciably to his expense, namely, clean pails, straining cloths, cans or bottles, and hands, a fairly clean place for milking, and a decent condition of the cow's udder and the adjacent belly, milk when first drawn will not average in hot weather over 30,000, and in cold weather not over 25,000 bacteria per cubic centimetre. Such milk, if cooled to and kept at 50° F., will not contain at the end of twenty-four hours over 100,000 bacteria per cubic centimetre. If kept at 40° F. the number of bacteria will not be over 100,000 per cubic centimetre after forty-eight hours.

If, however, the hands, cattle, and barns are filthy, and the pails are not clean, the milk obtained under these conditions will, when taken from the pail, contain very large numbers of bacteria, even up to a million or more per cubic centimetre.

Freshly drawn milk contains a slight and variable amount of bactericidal substances which are capable of inhibiting bacterial growth. At temperatures under 50° F. these substances act efficiently (unless the milk is filthy) for from twelve to twenty-four hours, but at higher temperatures their effect is very soon completely exhausted, and the bacteria in such milk will then rapidly increase. Thus the bacteria in fresh milk which originally numbered 5000 per cubic centimetre decreased to 2400 in the portion kept at 42° F. for twenty-four hours, but rose to 7000 in that kept at 50° F., to 280,000 in that kept at 65° F., and to 12,500,000,000 in the portion kept at 95° F.

As we have seen, the milk in New York City is found on bacteriological examination to contain, as a rule, excessive numbers of bacteria. During the coldest weather the milk in the shops averages over 300,000 bacteria per cubic centimetre, during cool weather about 1,000,000, and during hot weather about 5,000,000. The milk in other large cities is, from all accounts, in about the same condition.

The above statement holds for milk sold at the ordinary shops, and not that of the best of the special dairies, where, as previously stated, the milk contains only from 10,000 to 30,000 bacteria, according to the season of the year.

The question might be raised, Are even these enormous numbers of bacteria in milk during hot weather actually harmful?

Our knowledge is probably as yet insufficient to state just how many bacteria must accumulate to make them noticeably dangerous in milk. Some varieties are undoubtedly more harmful than others and we have no way of restricting the kinds that will fall into milk, except by enforcing cleanliness. We have also to consider that milk is not entirely used for some twelve hours after being purchased, and that during all this time bacteria are rapidly multiplying, especially where, as among the poor, no provision for cooling it is made. Slight changes in the milk which to one child would be harmless, would in another produce disturbances which might lead to serious disease. A safe conclusion is that no more bacterial contamination should be allowed than it is practicable to avoid. Any intelligent farmer can use sufficient cleanliness and apply sufficient cold, with almost no increase in expense, to supply milk twenty-four to thirty-six hours old which will not contain in each cubic centimetre over 50,000 to 100,000 bacteria, and no milk containing more bacteria should be sold.

The most deleterious changes which occur in milk during its transportation are now known not to be due to skimming off the cream, or to the addition of water, but to the changes produced in the milk by multiplication of bacteria. During this multiplication, acids and distinctly poisonous bacterial products are added to the milk, to such an extent that much of it has become distinctly deleterious to infants and invalids. It is the duty of health authorities to prevent the sale of milk rendered unfit for use through excessive numbers of bacteria and their products.

The culture tests to determine the number of bacteria present in any sample of milk require at least forty-eight hours; so that the sale of milk found impure cannot be prevented. It will, however, be the purpose of the authorities gradually to force the farmers and the middlemen to use cleanliness, cold, and dispatch in the handling of their milk, rather than to prevent the use of the small amount tested on any one day.

If the milk on the train or at the dealer's were found to contain excessive numbers of bacteria, the farmers would be cautioned and instructed to carry out the simple necessary rules, which would be furnished.

Transmission of Contagious Diseases through Milk.—No farmer or dairyman should allow anyone who has a contagious disease, or who

has been in contact with any person having scarlet fever, typhoid fever, measles, diphtheria, or consumption, to have access to the cattle, or to have any connection with the milk or milking, or with the milk utensils. Epidemics and outbreaks of contagious disease are often produced through the infection of the milk in this way. Every year epidemics occur which have been traced to milk contaminated by ignorant or careless milkmen, who have infected their milk from their dirty hands or the dirty water, or in other careless ways. This, of course, is entirely unnecessary and can be prevented. The extent of this danger may be judged by the fact that two years ago there was published in one of the medical journals a report upon 330 outbreaks of epidemic diseases traced to milk; 195 of these were epidemics of typhoid fever, in 147 of which the disease prevailed at the dairy or farm; in 67 it was due to contamination of well-water; in 24, employés at the farm were acting as nurses, and in 10 they were working while still sick. There were 99 epidemics of scarlet fever, in 68 of which the source of infection was traced to the illness of persons at the dairy; in 17 the employés were themselves suffering from scarlet fever, and in 10 they were acting as nurses to scarlet fever patients. In other cases the mode of infection was through the storage of milk near infected rooms, or the poison was brought by cans or bottles from patients' houses. There were 36 epidemics of diphtheria, in 13 of which the disease existed at the farm or dairy.

PART III.

PROTOZOA.[1]

CHAPTER XXXVII.

CLASSIFICATION AND GENERAL CHARACTERISTICS.

ALL animal forms consisting throughout their entire life of a single cell or of a colony of single cells are called protozoa.

They are so closely related to the protophyta, or lowest plant forms, on the one side, and the metaphyta, or many-celled animals, on the other, that it is difficult to mark out a sharp line of distinction between them.

In general, it may be said that each cell consists of protoplasm which is differentiated into nucleus and cytoplasm, both parts showing many variations in the several groups, and that each cell undergoes a more or less complicated life cycle, appearing in different forms at different stages of development.

Doflein, in Kolle and Wassermann, gives the grouping of the protozoa as follows:

PHYLUM: PROTOZOA.

- I. Subphylum: Plasmodroma. Doflein.
  - I. Class: Rhizopoda. v. Siebold.
    - I. Order: Amœbina. Ehrenberg.
    - II. Order: Heliozoa. Haekel.
    - III. Order: Radiolaria. Johannes Müller.
    - IV. Order: Foraminifera. d'Orbigny.
    - V. Order: Mycetozoa. de Bary.
  - II. Class: Mastigophora. Diesing.
    - I. Subclass: Flagellata. Cohn em. Bütschli.
      - I. Order: Protomonadina. Blochmann.
      - II. Order: Polymastigina. Bütschli and Blochmann.
      - III. Order: Englenoidina. Klebs.
      - IV. Order: Chromomonadina. Blochmann.
      - V. Order: Phytomonadina. Blochmann.
    - II. Subclass: Dinoflagellata. Bütschli.
      - I. Order: Adinida. Bergh.
      - II. Order: Dinifera. Bergh.
    - III. Subclass: Cystoflagellata.
      - Appendix: Trichonymphidæ. Leidy.

[1] The following general authorities on protozoa have been consulted freely in this work: Doflein and Prowazek, in Kolle and Wassermann; Minchin, in Ray Lankester; Calkins.

PHYLUM: PROTOZOA.

III. Class: Sporozoa. Leuckart.
  I. Subclass: Telosporidia. Schaudinn.
    I. Order: Coccidiomorpha. Doflein.
      I. Suborder: Coccidia. Leuckart.
      II. Suborder: Hæmosporidia. Danilewski em. Schaudinn.
    II. Order: Gregarinida. Aimé Schneider em. Doflein.
      I. Suborder: Eugregarinida. Doflein.
      II. Suborder: Amœbosporidia. Aimé Schneider.
  II. Subclass: Neosporidia. Schaudinn.
    I. Order: Cnidosporidia. Doflein.
      I. Suborder: Myxosporidia. Bütschli.
      II. Suborder: Microsporidia. Balbiani.
    II. Order: Sarcosporidia. Balbiani.
      Appendix: Serumsporidia, Haplosporidia, Lymphosporidia.
II. Subphylum: Ciliophora. Doflein.
  I. Class: Ciliata.
    I. Order: Holotricha. Stein.
    II. Order: Heterotricha. Stein.
    III. Order: Oligotricha. Bütschli.
    IV. Order: Hypotricha. Stein.
    V. Order: Peritricha. Stein.
  II. Class: Suctoria. Bütschli.

So far only a few of the protozoa have been shown to produce disease in man; in the lower animals the number of pathogenic forms is slightly larger, but the great majority seem to dwell as harmless parasites in the bodies of their hosts.

The groups which are of medical interest are the following: The amœbina[1] and the mycetozoa, from the class rhizopoda; the flagellata, from the class mastigophora; the sporozoa as a class, and one order from the ciliata.

The Amœbina (Fig. 144) include forms composed of naked, simply constructed protoplasm having the power of producing variously shaped pseudopodia, or protoplasmic processes, which are used as organs of locomotion and of nutrition. They possess generally one nucleus and a contractile vacuole.

The life cycle of only a few varieties has been studied, and until the full cycle of development of any so-called amœba is known it is impossible to say whether it belongs among the rhizopoda, or whether it is one of the forms of development of another group, as amœboid forms may occur at some time in the life history of all groups. According to the few forms studied, the amœbæ increase by (1) simple division and by (2) encysting. In the latter case the nucleus divides into many daughter-nuclei, which arrange themselves around the periphery,[2] and, under favorable conditions, the protoplasm divides about them and young amœbæ are formed, leaving often a central mass of protoplasm, or "crystal residuum" ("Rest Körper"). The young amœbæ then break through the cyst membrane and soon assume the forms of the

[1] See figures under amœba coli.

[2] See figures under amœba coli.

adult amœbæ. In one species, after division of the nucleus and protoplasm, the daughter-cells become "swarm spores" with two flagella. A certain form of conjugation has been observed in some varieties.

Amœbæ are found mostly in standing fresh water and sea-water and on moist vegetable substances. A few have been described in the body fluids of the higher animals. Those so far known to be parasitic for man are amœba coli and a few closely related varieties which are described below.

FIG. 140

B

A. Cell of root of cabbage infiltrated with plasmodiophora amœbæ. The amœbæ are fusing, forming plasmodia. *B*. Beginning mitotic division of the amœbæ. The nucleus of the host cell beneath. (After Nawaschin.)

The Mycetozoa are a group of organisms showing characteristics of both plants and animals. They are, therefore, claimed by botanists as well as zoölogists. They have possessed some interest of late from a medical standpoint because one variety, the plasmodiophora brassicæ Waronin (Figs. 140 and 141), which is an intracellular parasite of members of the cruciferæ, producing large tumors in their roots ("fingers and toes," "club-foot"), has been shown to form cell inclusions somewhat similar to some of the cell inclusions seen in the carcinomata, and certain investigators have thought that it bears a relation to the etiology of carcinoma. But so far no relationship has been proven. The plasmodiophora brassicæ, when inoculated into experimental animals, produces only small granulomata which finally disappear.

The Flagellata[1] are organisms composed of naked, variously constructed protoplasm, and moving by means of one or more long flagella. Some forms also show amœboid movements. The number and characteristics of the flagella vary. Generally there is a principal flagellum directed forward, and there are from one to several flagella directed backward. Often a flagellum seems to be attached throughout part of its length to the body of the organism by a wavy or undulating membrane. The protoplasm of the flagella appears homogeneous, except for the fact that it may occasionally show small granules. The flagella arise from small granules, the "flagella roots," which, according to some authors, are similar to centrosomes, and, according to others, micronuclei. The body of the flagellata is generally round or oval and more or less motile. Its protoplasm may be finely or coarsely granular, sometimes reticular, and may contain one or more vacuoles, one or two of which, situated anteriorly, may be contractile. It

FIG. 141

Two cells infiltrated with spores of the plasmodiophora brassicæ. (Doflein.)

may also contain various food particles. The nucleus situated anteriorly varies in appearance in the different species. It is usually more or less granular, with a central body, but in some species it is vesicular. The flagellata multiply either in the purely motile condition or after encysting. In the first case the division is generally longitudinal. In the second case they may or may not conjugate before they encyst. Then division forms occur in the cyst by a process somewhat similar to that in the amœbæ.

Flagellates are found in foul and stagnant water, in the ocean, and a few in rivers and in the body fluids of higher animals. The following species which are described below have been reported as being parasitic in man: trypanosoma, cercomonas, trichomonas, lamblia intestinalis.

The sporozoa are a group of exclusively parasitic protozoa of very widespread occurrence, living in the cells, tissues, and cavities of ani-

[1] See Figs. 146 and 147, under trypanosoma Lewisi.

mals of every class. Generally they are harmless, but some varieties may produce pathological changes and even fatal diseases severely epidemic.

As their name indicates, they are all characterized by reproduction through spore formation, but they exhibit the utmost diversity of structural and developmental characteristics. As a rule, each species is parasitic on one kind of tissue of a particular species of host. They are generally taken into the system in the spore stage either (1) with the food of the host, (2) by the bites of insects, or (3) by inhalation. The spore membranes are dissolved by the fluids of the host, and thus one or more germs or sporozoites are set free to bore into the special cells of the host. Here they grow, some remaining intracellular permanently,

FIG. 142

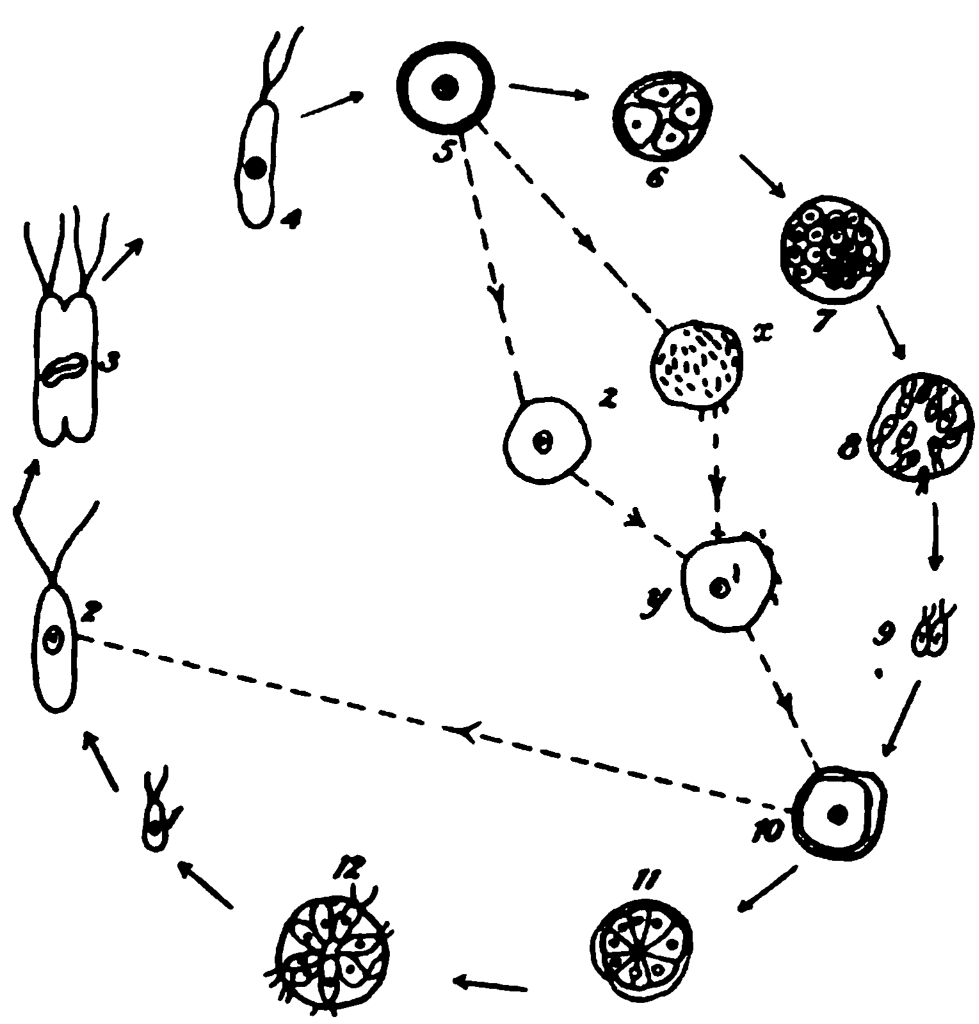


Diagram of variations in life cycle of flagellates: 1, a young flagellate; 2, adult flagellate; 3, longitudinal division of adult free form; 4, daughter flagellate; 5, encystation; 6–8, division into isogametes; x and z, division into macrogametes and microgametes, characteristic for some forms; 9, conjugation of the isogametes; y, conjugation of the macrogametes and microgametes; 10, resting-stage—zygote; 11–12, division into young. (After Doflein.)

others only in the young stages. The latter either pass different phases of their more or less complicated life history in different parts of the body of one and the same host, or they pass some phases of their life cycle in the cells of an intermediate host.

The sporozoa vary widely in size as well as in other characteristics. From the smallest, several of which can be contained in a single blood cell, there are all gradations in size up to those that may be seen by the naked eye (Prospora gigantea, 16 mm.).

Besides being characterized by the power to produce more or less resisting spores, the sporozoa are also characterized by the fact that as a class they possess none of the special organs found in other protozoa for ingesting or digesting solids. Many develop flagella or show amœboid

movement during certain stages of their life cycle, but the flagella and pseudopodia are organs of locomotion and not of nutrition except in

Fig. 143

so far as they increase the absorptive surface of the body, as food is absorbed by diffusion. Food vacuoles or contractile vacuoles have not been found.

The life cycle of a typical sporozoan is represented after Schaudinn in Fig. 143.

A somewhat similar cycle may be followed in the study of the coccidium cuniculi of the rabbit, a description of which is given below. The other varieties in this group, which are parasitic in man, or which are of some medical interest, and which are described below, are the following:

Various not fully studied coccidia.
Plasmodium malariæ and its allies.
Piroplasma bigemmium and its allies.
Nosema bombycis and nosema lophii.

The Ciliata (See Fig. 159) belong to the most complex of the protozoa. They possess a definite entoplasm containing nuclei and food vacuoles, and a definite ectoplasm containing basal granules from which arise the cilia which give the group its name. They have organoid structures which receive the food, and some have definite mouth openings indeed, and definite places for excreting waste products. The food vacuoles may contain acid or alkaline digestive products. The nuclear material is differentiated into two forms, a large macronucleus and a much smaller micronucleus. The function of the macronucleus is supposed to be vegetative, and that of the micronucleus reproductive. The macronucleus varies in size and shape and is completely filled with an alveolar

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Description of Fig. 143.

The life cycle of coccidium Schubergi. *I* to *VII* represent the asexual reproduction or schizogony, commencing with infection of an epithelial cell by a merozoite or a sporozoite; the merozoite after stage *VII* may start again at stage *II*, as indicated by the arrows, or it may go on to the formation of gametocytes (*IX* to *XII*). *IX* to *XIV* represent the sexual generation, the line of development becoming split into two lines—male ♂ and female ♀, culminating in the highly differentiated gametes, which conjugate and become again a single line, shown in *XIV* and *XV*. The zygote thus formed goes on to the production of spores, *XVI* to *XX*. *I* to *IV* represent epithelial cells showing penetration of a merozoite or a sporozoite and its change into a schizout. *V*, the nucleus of the schizout dividing. *VI*, numerous daughter-nuclei in the schizout. *VII*, segmentation of the schizout into numerous merozoites, about a central mass of residual protoplasm, which in this figure is hidden by the merozoites. *VIII*, merozoite passing to reinfect host cell and repeat the process of schizogony. *IX*, *X*, merozoites to be differentiated into male and female gametocytes. *XIa* and *XIIa*, the two gametocytes within a host cell; the microgametocyte (♂) has fine granulations; the macrogametocyte (♀) has coarse granulations. *XIb*, an immature female gametocyte within a host cell. *XIc*, a female gametocyte undergoing maturation, still in the host cell. *XIII*, mature macrogametocyte, freed from the host cell, and sending a cone of reception toward an approaching microgametocyte. *XIIb*, a full-grown micro-gametocyte within a host cell. In *XIIc* the nucleus of the microgametocyte has divided up to form a great number of daughter-nuclei. In *XIId* the nuclei of the last stage have become microgametes, each with two flagella. *XIIe*, represents the free microgametes, swimming to find a macrogamete. *XIV*, the zygote (fertilized macrogamete), surrounded by a tough membrane or oöcyst, which allows no more microgametes to enter, and containing the female chromatin, which is taking the form of a spindle, and the male chromatin in a compact lump. *XV*, the chromatin from these two sources united and no longer distinguishable as male and female. *XVI*, the nucleus of the zygote dividing. In *XVII* four daughter-nuclei are formed—the nuclei of the sporoblasts. In *XVIII* the four sporoblasts become distinct, leaving a small quantity of residual protoplasm; each sporoblast has formed a membrane, the sporocyst. In *XIX* within each sporocyst two sporozoites have been found about a sporal residuum. In *XX*, the sporozoites becoming free by bursting the sporocysts, pass out through an aperture, in the wall of the oöcyst, and are ready to enter the epithelial cells of the host. (From Lang.)

chromatin. The micronucleus also varies in size and shape but is vesicular in structure, with the chromatin heaped in one mass. Division of the nuclei takes place by mitosis in the case of the micronuclei, and by amitosis, as a rule, in the case of the macronuclei. Under unfavorable conditions the ciliata may encyst.

Conjugation is necessary to the life activity of these organisms. The phenomena of conjugation in the ciliata has been well worked out. The micronuclei play the most important part, whereas the macronuclei simply break up and disappear in the protoplasm.

According to the arrangement of the cilia the ciliata are divided into the five orders given in the general classification. Among these the second, the order of the Heterotricha, interests us. In the Heterotricha the cilia are uniform over most of the body, while a specialized set fused into a series of firm vibratory plates are found about the mouth.

Only one genus—the Balantidium, described in a later chapter—has been observed in man.

A description is also given of various protozoan-like forms found in the following diseases: smallpox, cowpox, and related diseases; scarlet fever, measles, kala-azar, and hydrophobia.

CHAPTER XXXVIII.

ORDER: AMŒBINA.

Amœba coli (Lösch).

SEVERAL varieties of amœbæ have been reported as occurring in the human intestines, but as the full cycle of no one of them has been worked out it is possible that some of them at least may be stages in the life cycle of other protozoa. The most important one among these varieties is that observed by Lambl in 1860, and more fully described by Lösch in 1875 as amœba coli. Lösch found it in the stools of dysenteric patients, and he succeeded in producing, experimentally, superficial ulcerations in the large intestines of dogs. He therefore claimed that this organism is the cause of dysentery.

His work was corroborated by many observers, the organism being also found pure in tropical abscess of the liver; but later studies have shown that this parasite does not play as important a part in dysentery as was thought. It has been found that in many cases of dysentery the amœba coli is not present, that the same or similar amœbæ are present in the healthy stools, and that animal experiments are not satisfactory. These facts, together with those brought out by the recent work of Shiga, Kruse, Flexner, and others, on bacillary dysentery, have demonstrated that there are at least two forms of dysentery, one produced by amœbæ and the other by bacilli, and that in the former case, among a number of harmless varieties of intestinal amœbæ, one or two only are of clinical importance. According to Shiga the differential diagnosis between the two varieties of dysentery is as follows:

In amœbic dysentery (1) the disease is generally chronic; (2) no dysentery bacilli are found in the feces; (3) there are not present any severe toxic symptoms, such as fever (except in the case of abscess of the liver), weakness, headache, anorexia, rapid emaciation, hemorrhages, etc.; (4) abscess of the liver is a frequent sequela; (5) the lesion is in the cæcum and descending colon; the small intestines are not affected.

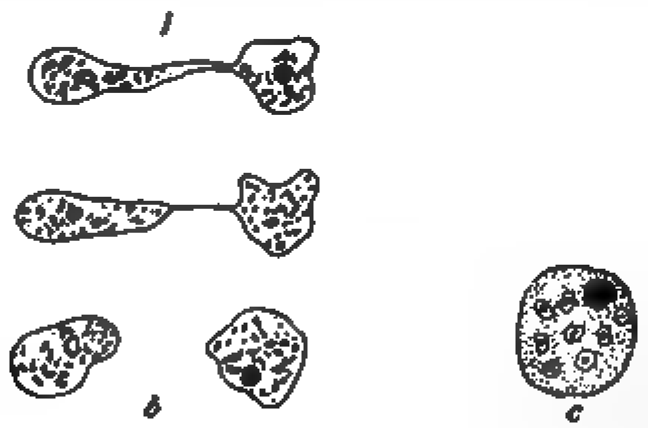
In bacillary dysentery the finding of the bacilli and the positive results of agglutination tests, together with the clinical symptoms of intoxication, make a certain diagnosis.

At present we may group the pathogenic amœbæ under one head, calling the groups amœba coli; though Schaudinn, in his recent work on the amœbæ of the human intestines, has given quite a definite description of two varieties, one of which he calls entamœba coli Lösch, which is a harmless comensal in the human intestines, and the other entamœba

hystolytica Schaudinn, which corresponds with the form studied by Councilman and Lafleur and by Jürgens, and which is pathogenic, producing the true amœbic dysentery. He says that the pathogenic variety has a more definite ectoplasm than the non-pathogenic; furthermore, that its nucleus contains less chromatin, making it more difficult to demonstrate, it has a more varied form and is always situated eccentrically. This variety increases by division into two and by budding, while the non-pathogenic variety increases by a regular division into eight daughter forms (schizogony). In unfavorable conditions both varieties may produce cysts.

Morphology.—The size of the amœba coli is given variously by different authors within the limits of 7μ to 50μ, more commonly 12μ to 30μ. In a state of rest it assumes a spherical shape which appears discoid under the microscope. It may generally be distinguished from the other cellular elements found in the feces by its pale-greenish tint and by its stronger refraction of light. The outline of the body ordinarily appears as a thin, single, dark line. The two portions of the body, the inner, or

FIG. 144



Amœba coli. *a.* Containing red blood cells (Römer). *b.* Mode of division (Harris). *c.* Cyst with eight nuclei (Grassi).

entoplasm, which is more or less granular and of a darker color, and the outer, or ectoplasm, which is homogeneous and of a lighter color, cannot always be made out and are more evident in the motile than in the resting amœba.

The entoplasm constitutes the greater portion of the body of the amœba. In the smaller forms it is finely granular, and may show no other structure. In the larger forms it is more coarsely granular and may contain, in varying numbers, bacteria, starch bodies, cell detritus, and red and white blood cells, in various stages of digestion. One to several vacuoles have been seen in the entoplasm, but only Dock has spoken of their infrequent pulsation.

The ectoplasm forms a hyaline zone of variable thickness about the entoplasm. It has the appearance, under the microscope, of finely ground glass of a distinctly pale-green color. It seems often to pass out into short, irregular pseudopods.

The nucleus of the amœba is 2μ to 7μ in diameter. It is a more or less spherical vesicle-like body containing a dark, chromatin inner

body which is connected with the thick nuclear membrane by a protoplasmic network.

It is not always easy to detect the nucleus in fresh or motile amœbæ, but under certain conditions in motionless or dead amœbæ it becomes evident. It may be easily shown by appropriate staining reagents.

Biological Characters.—The most striking and characteristic feature of the amœba is its motility. This may consist either in an alteration of its shape or in an actual change of place. Both of these phenomena are produced through the mechanism of pseudopodia. These latter are rounded, blunt, and homogeneous processes formed by the more or less gradual protrusion of a portion of the ectoplasm at some part of the periphery of the amœba. The motion is at times quite gradual and continuous, at others sudden and jerky. The progressive movement, that is, actual locomotion, is brought about by the protrusion of pseudopodia, and into these, when they have reached a certain size, the more or less granular and vacuolated entoplasm, with its contents, flows with a more rapid movement than that by which the pseudopodia themselves were formed. Locomotion is generally observed to take place in the direction of least resistance, a group of cellular elements or some detritus being sufficient to divert the course of the amœba. The amœboid movements are also influenced by various other factors, particularly by variations of temperature. They are most active at the mean temperature of the human body, becoming less active as the temperature falls or rises above this mean, and indeed they become quite motionless in a temperature lower than 75° F. According to Boas, amœbæ remain alive outside of the body for not more than twenty-four hours.

Sexual reproduction has been described by Schaudinn for some forms; but the usual modes of multiplication are by simple division and by division after cyst formation. Attempts to cultivate amœbæ outside of the body have been unsuccessful until recently, when Musgrave and Clegg described the cultivation of pure species in pure cultures of bacteria—the "pure mixed cultures" of Frosch.

Animal Experiments.—It is evident that in the absence of artificially produced pure cultures of amœbæ, inoculation experiments must be made with material such as dysenteric stools or the contents of hepatic abscesses. In a few cases where material has been obtained from hepatic abscesses which have been found to contain no organisms other than amœbæ, the inoculations have been made in three ways: (1) by feeding animals with material containing the amœbæ; (2) by inoculation into the small intestines after a preliminary laparotomy; and (3) by rectal injections with or without suture of the anal orifice. The first method has always proved unsuccessful, except when encysted forms were present. To the second method the objection has been raised that the manipulation of the intestines and the use of antiseptic solutions during the course of the operation are in themselves a source of irritation to the bowel and in some cases have produced an enteritis. The third method has given, though not in every case tried, positive results in the hands of Lösch, Kruse, Pasquale, Jürgens, and others. In the successful cases the lesions found

were reddening and swelling of the intestinal mucosa, chiefly of the lower half of the large bowel, with here and there ecchymoses, small superficial areas of necrosis, and shallow ulcerations. The mesenteric glands and the solitary lymphoid follicles were often swollen. In the blood-tinged mucus covering the mucous membranes amœbæ were found in greater or less numbers. Microscopic examinations showed that the necrosis was limited as a rule to the mucosa, and that beneath it the submucosa was thickened and œdematous and its vessels engorged; there was also small-celled infiltration. Amœbæ were found in the borders of the ulcers, chiefly in the follicles of Lieberkühn; in the base of the ulcers they rarely penetrated more deeply than the upper layers of the submucosa. With the amœbæ were found many bacteria, chiefly streptococci.

Concerning the source of the amœbæ and the mode of infection little can be positively stated. It is reasonable to suppose, however, that

FIG. 145

Leydenia gemmipara Schaudinn. *A*, single amœba; *B*, plasmodia and budding; *n*, nucleus; *n'*, nucleus dividing; *cv*, contractile vacuole; *v*, vacuole; *er*, red blood cell; *Kn*, buds; *Ka*, amœba developed from bud.

the mouth must be the usual path of infection, and that the amœbæ in all probability are taken in with the drinking-water and with uncooked vegetables.

Methods of Examination.—The stools must be examined in as fresh a condition as possible. The bloody masses of mucus and the material about them should be chosen to be examined. It may be necessary to thin the particles examined with physiological salt solution. The warm stage should be used and the cover-glass should be supported by small feet of sealing wax. The collected material should be kept at body temperature until time of examination.

For permanent preparations Jäger especially recommends the following method: For a fixative a concentrated watery solution of sublimate 100 c.c., absolute alcohol 50 c.c., acetic acid 5 drops. After a few minutes' fixation wash carefully with iodine-alcohol, then stain with Grenacher's hæmatoxylin ten minutes, afterward wash with water

until no more blue stain comes from the specimen, then stain with 1 per cent. eosin. The nuclei of the leukocytes are stained blue, while those of the amœbæ are stained red.

Mallory and Wright recommended the following method for sections containing amœba coli: 1. Harden in alcohol. 2. Stain sections in a saturated aqueous solution of thionin three to five minutes. 3. Differentiate in a 2 per cent. aqueous solution of oxalic acid for one-half to one minute. 4. Wash in water. 5. Dehydrate in alcohol. 6. Clear in oleum origani cretici. 7. Wash off with xylol. 8. Xylol balsam.

Amœbæ in Diseases Other than Dysentery.—Kartulis reported finding a large motile amœba (30μ to 38μ) in an abscess of the lower jaw of an Arabian. Flexner also observed a similar case in a sixty-two-year-old man. Baelz found a very large amœba in the bloody urine and in the vagina of a twenty-three-year-old Japanese who was suffering from tuberculosis of the lung. Jürgens, Kartulis, and Posner also reported finding similar amœbæ in cases of cystitis and bloody urine.

In the ascitic fluid of a man who had carcinoma of the stomach Leyden found motile cellular elements which Schaudinn pronounced independent organisms belonging to the rhizopoda Leydenia gemmipara (Schaudinn), Fig. 145. Similar organisms were found in the ascitic fluid of a girl who had an abdominal tumor. The organisms remained motile in the ascitic fluid seven days after its removal. The organism possesses a pulsating vacuole and one vesicular nucleus; it divides directly and by budding. The individuals seem readily to fuse (plastogamy). The pathological significance of this rhizopod is not clear.

CHAPTER XXXIX.

TRYPANOSÓMA.

Subclass: Flagellata.
Order: Protomonadina.
Genus: Trypanosoma.[1]

The genus trypanosoma includes blood parasites of the vertebrates distinguished by a somewhat long body more or less spirally twisted, one to several flagella, an undulating membrane, one nucleus, and a "flagellum root." The chief flagellum is directed forward, arising from or near a small, more or less rounded structure, the "flagellum root" (blepharoplast), situated near the posterior end of the organism. The nature of the "flagellum root" is still a matter of controversy. Some consider it of the nature of a centrosome, others, of that of a micronucleus. Schaudinn calls it the locomotor nucleus, and says it produces undulatory membrane and flagella. If secondary flagella are present they are generally directed backward.

The undulating membrane extends along one side of the organism from the "flagellum root" or near it to the anterior end of the parasite, whence it continues as the free flagellum. It varies in size and fulness according to age and species.

The nucleus is situated anteriorly; it is granular, thick, and egg-shaped, but varies somewhat in size and shape. The cytoplasm is homogeneous or granular, varying with age, environment, and possibly species. The life cycle is not well known. Multiplication occurs through longitudinal division; however, MacNeal states that the division is not exactly longitudinal, but always more or less oblique in direction, and that the flagellum does not divide, but a new one is formed in each division. Conjugation has been observed in trypanosoma Brucei by Plimmer and Bradford.

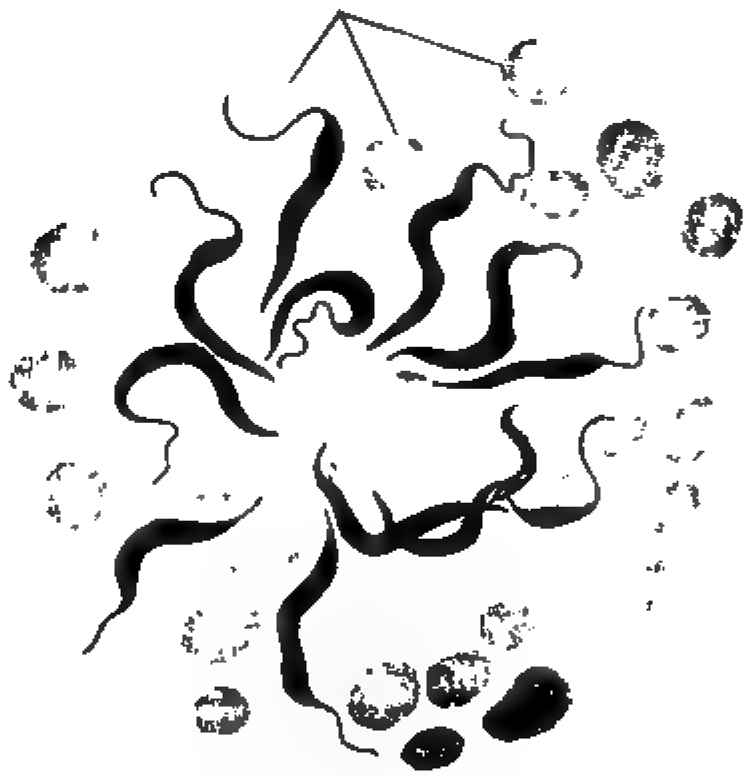
Only a few of the many species of trypanosomes described are pathogenic, and these principally for the lower animals, though recently the organisms have several times been seen in human beings, accompanied or not by pathological changes. Very recently Castellani stated that the sleeping sickness of the negro is caused by a trypanosome. The work of Schaudinn and of Novy and MacNeal make it evident that the spirochætes of recurrent fever and of geese belong to the trypanosomes.

The first species of trypanosomes studied with any degree of fulness is the comparatively non-pathogenic trypanosoma Lewisi, Kent. It is of interest because of its similarity to the more pathogenic forms and because of the ease with which it may be studied. It is parasitic in the blood of

[1] Special literature on trypanosoma: Laveran and Mesnil, Musgrave and Clegg, Novy and MacNeal, and Schaudinn.

about 25 per cent. of wild rats in almost all parts of the world. It is found less frequently in tame rats, especially in the white variety. It occasionally

Fig. 146



Trypanosoma Lewisi (Kent). From the blood of a rat. (Kempner and Rabinowitsch.)

Fig. 147

Agglutination of Trypanosoma Lewisi. (Laveran and Mesnil.)

produces sickness and death and even small epidemics, but generally it is found in apparently healthy animals. A morphologically similar trypanosome is found in hamsters, but as neither variety will grow in

the blood of the other host they must be regarded as physiological varieties.

These flagellata were probably first seen in the blood of the rat in 1845, but they were not well described until 1879, when Lewis studied them more fully. Since then they have been studied by many observers, especially by Kempner and Rabinowitsch, Wasielewski and Senn, Jürgens, Laveran and Mesnil, and Novy and MacNeal.

Morphology.—Their length, including the flagellum, is from 8μ to 30μ, their breadth 2μ to 3μ. The body is lance-shaped and shows a protoplasm finely granular in the young form, more coarsely so as age increases. The single flagellum is almost as long as the body and arises from the posterior third of the organism in or near a small, more or less oval body, the flagellum root (centrosome, micronucleus blepharoblast), which during division often divides first. The flagellum continues forward as the thickened edge of the undulating membrane, becoming free at the anterior end of the body. The large, oval, densely reticular nucleus lies generally in the anterior third of the body. No contractile vacuoles have been observed.

Biology.—The parasite is very motile, probably more so than any other variety. Its motility soon ceases outside of the body, continuing longer in the ice-box than at higher temperatures. Also unless kept at low temperatures the organism dies very quickly. It is rather diagnostic of it that at ice-box temperature it lives longer than any other variety of trypanosome studied. Long after the organisms have lost their motility and ability to stain well, and even after they seem to have broken up completely, the blood containing them is still infectious for rats. In this connection it is interesting to note that the blood of infected animals in which no trypanosoma can be demonstrated is infectious for fresh animals.

Kempner and Rabinowitsch have succeeded in producing active and passive immunity. The blood of immunized animals causes agglutination of the trypanosomes without immobilization. According to Laveran and Mesnil the serum possesses no lytic properties for the trypanosomes, and they state that the inoculation of such serum intraperitoneally with active trypanosomes seems simply to cause an increased power of the phagocytes over them, whereas MacNeal states that the serum does possess cytolytic properties for the parasites.

Recently, Novy and MacNeal have reported the artificial cultivation of the rat trypanosome. At room temperature they have grown the organism through eleven culture generations in test-tubes for an entire year. At the end of this time the parasites were as virulent as at the beginning. The culture medium used in their work was ordinary nutrient agar containing variable amounts of fresh defibrinated or laked rabbit or rat blood. The best results were obtained with a mixture of two parts of the blood to one of agar, though growth was obtained on dilutions as high as one part of blood to ten of agar. At room temperature the growth is slower but surer than in the thermostat. The cultures at room temperature retain their vitality for months; thus in one case the trypanosomes were alive after three hundred and six days.

These results have been corroborated by Kempner and Rabinowitsch, Laveran and Mesnil, and by ourselves.

Trypanosoma Evansi (Steel).

The next trypanosome of importance studied, and the first of the more pathogenic trypanosomes, is trypanosoma Evansi, Steel. This species was discovered by Evans in 1880 in the blood of horses suffering with the disease known as surra, in India. Lingard's important work on this disease in 1893 led, in a way, to all the subsequent work on diseases caused by trypanosomes. In general the descriptions given of the symptomatology of trypanosomiasis in various animals show a great similarity, though there is much variation in individual cases. In a well-established infection the clinical picture according to Musgrave and Clegg is as follows: After an incubation period which varies in the same class of animals and in those of different species, as well as with the conditions of infection, and during which the animal remains perfectly well, the first symptom to be noticed is a rise of temperature; and for some days a remittent or intermittent fever may be the only evidence of illness. Later on the animal becomes somewhat stupid; watery, catarrhal discharges from the nose and eyes appear; the hair becomes roughened and falls out in places. Finally the catarrhal discharges become more profuse and the secretions more tenacious and even purulent; marked emaciation develops; œdema of the genitals and dependent parts appears; a staggering gait, particularly of the hind parts, comes on, and is followed by death. There may be various ecchymoses and skin eruptions. Parasites are found in the blood more or less regularly after the appearance of the fever.

The autopsy generally shows anæmia, an enlarged spleen with hypertrophied follicles, more or less gelatinous material in the adipose tissue, the liver slightly enlarged, a small amount of serous exudate in serous cavities, œdematous condition, and small hemorrhages in various tissues.

The duration varies from a few days to many months. The prognosis seems to be influenced to a certain extent by the species of host. It is probably always fatal in horses. Some cattle recover. The cause of death is possibly a toxic substance, though no definite toxin has been isolated. Mechanical disturbances (emboli, etc.) also probably play a part in producing death. The hosts of trypanosoma Evansi are horses, mules, cattle, camels, elephants, buffaloes, and, according to Musgrave and Clegg, rats. After experimental inoculation this trypanosome is infectious for dogs, monkeys, rabbits, guinea-pigs, mice, and cats. Man seems to be immune. It is without doubt transmitted from animal to animal by the bites of insects (flies and fleas).

Besides the differences in virulence, the trypanosoma Evansi is differentiated morphologically from the trypanosoma Lewisi by a larger average length (20μ to 30μ long and 1μ to 2μ wide). It differs from the trypanosoma Brucei in having a more pointed posterior end. Many authors, however, consider it identical with the latter species.

Trypanosoma Brucei (Plimmer and Bradford).

The trypanosoma Brucei (Plimmer and Bradford) was discovered by Bruce in 1894 in the blood of horses and cattle suffering from nagana in Zululand and other parts of Africa. Bruce demonstrated that the contagion was caused by the bites of a fly, the *Glossina morsitans*, or tsetse-fly. Since then other varieties of flies also have been shown to spread the disease. These flies bite by day and in full moonlight. The infectivity of the insects lasts for about forty-eight hours after they have bitten a sick animal. Bruce found living trypanosomes in the proboscedes of the flies at the end of that time. Up to one hundred and eighteen hours they were found in the flies' stomachs, but after one hundred and forty hours the stomachs were empty and what appeared to be dead parasites were found in the excreta. No development in the fly has been observed. The disease is chronic enough in some animals to account for a continuous source of infection. The natural hosts of this species are horses, cattle, camels, antelopes, swine, and various wild animals. According to Laveran and Mesnil all mammifera are susceptible to trypanosoma Brucei, though sheep and African goats seem to be partial exceptions. Men and birds seem to be immune. Horses and dogs are especially susceptible. The incubation time in natural infection is not more than nine days. The course and duration are irregular, as in other trypanosomatic diseases.

Novy and MacNeal have been successful also in cultivating the trypanosoma Brucei *in vitro*, though it is much more exacting in its requirements than is the trypanosoma Lewisi. The same methods are used, but the blood dilution must not be less than two parts to one of nutrient agar. These investigators state that the cultural characteristics are such as to enable perfect differentiation between the two trypanosomes. For in their cultures the trypanosoma Brucei have characteristic granules, the trypanosoma Lewisi have none; the trypanosoma Brucei show little variation in size (15μ to 17μ in length), the trypanosoma Lewisi vary so much (1μ to 60μ long) that there are forms small enough to pass a Berkefeld filter; the trypanosoma Brucei has a slow, wriggling motion, the trypanosoma Lewisi moves with great rapidity and in an almost straight line; and finally the trypanosoma Brucei form small, irregular colonies, while the trypanosoma Lewisi form large symmetrical ones.

The question as to the identity of the trypanosoma Brucei with other of the more pathogenic trypanosomes has not yet been decided.

So far it has not been possible to immunize the more susceptible animals against this species of trypanosome. Sheep, goats, and cattle are less susceptible and in their case recovery from the disease protects against subsequent inoculation.

Novy and MacNeal state that older cultures of trypanosoma Brucei, especially those exposed to a temperature of 34° C., become less virulent and eventually, though living, fail to infect animals, and they think that repeated injections of these attenuated cultures may

impart immunity, and that in this way it may be possible to secure protection against the ravages of nagana.

In 1896 Rouget discovered a trypanosome in the blood of bleeding equines in Algiers and South Africa affected by the disease called Dourine. Some authors think this disease is identical with nagana and surra, but the fact that it has been impossible to infect cattle with the blood of the sick equine point to its being a distinct disease. The trypanosoma is called trypanosoma equiperdum or trypanosoma Rougeti.

Recently, mal de caderas, a disease of horses, asses, and mules in South Africa, having the general characteristics of trypanosomiasis, has been shown by Voges to be due to trypanosomes. He called this organism trypanosoma equinum, and believes it to be a distinct species, resembling more closely the trypanosoma Lewisi. He considers cattle immune.

In 1902 Laveran described a variety of trypanosome found by Theiler to produce disease in ruminants of the Transvaal. According to Laveran and Mesnil it is characterized by having the "flagellum root" near the centre of the parasite, near and sometimes united to the nucleus. This variety has been named trypanosoma Transvaaliense, while another variety found in cattle of South Africa by Theiler, and pronounced by Laveran and Mesnil to be a distinct species, was named trypanosoma Theileri.

Trypanosomes have been found in the blood of apparently normal frogs, fishes, birds, guinea-pigs, rabbits, and bats. No relationship has been shown to exist between these non-pathogenic forms and those causing disease in the higher animals.

Trypanosomes in Man.

In 1898 Nepvieu reported having found trypanosomes in the blood of 6 out of more than 200 cases examined for malarial organisms. In all of these cases malarial organisms too were found, and no symptoms characteristic of the invasion of trypanosomes were observed. Nepvieu found flagellates in a seventh case which was apparently in good health.

The eighth case is reported by Dutton in 1901. This case was a European who had lived some years in West Africa. The principal symptoms were gradual wasting and weakness; irregular temperature, never very high and of a relapsing type; local œdemas, congested areas of the skin, enlargement of the spleen, and constant increased frequency of pulse and respiration. It ended fatally after one year and eight months. The chronic character was repeated in animals. Some white rats were refractory; others died in two to three months. In monkeys (*Macacus rhesus*) it was fatal in about two months. Dogs were unaffected. This trypanosome is distinctly smaller than the other species described, and there is little doubt of it being a distinct species. Dutton also found trypanosomes in the blood of 1 out of 150 apparently healthy Gambian children examined by him.

The tenth case is published by Manson in 1902. This was a mis-

sionary's wife who had resided on the Upper Congo for a year. She presented the same group of symptoms as Dutton's case, and after repeated examinations trypanosomes were found in her blood. Manson soon after published a similar case. Broeden has published 2 more cases and recently Baker has reported 3 cases among human beings.

Of these 16 cases of trypanosomiasis in man, 2 were apparently healthy persons, 6 had malarial fever as well, and 8 were such as showed clinical symptoms apparently entirely due to the infection with trypanosomes.

Quite recently Castellani has stated that the cause of sleeping sickness of the negro is a trypanosome. He found trypanosomes in the centrifugalized cerebrospinal fluid of 20 out of 34 cases of this disease. His work has been corroborated by Bruce, Nabarro, Greig and others. Bruce found trypanosomes in the fluid obtained by lumbar puncture in all of the 38 cases examined and in 12 out of 13 cases in the blood. The trypanosomes found in these cases resemble those found in other human beings, and probably belong to the same species. Laveran and Mesnil recommend the names trypanosoma Gambiense Dutton for the parasite and human trypanosomiasis for the disease.

Sleeping sickness, or human trypanosomiasis, is a disease of the negro, endemic in certain regions of equatorial Africa. Neither age nor sex are predisposing factors, but occupation and social position seem to have a marked influence, the great majority of the cases occurring among very poor field workers. As these workers are all negroes, the question of the influence of race cannot be determined. The white race, however, is not immune, as has been shown by the cases quoted above.

In places where most of the cases occur, a fly belonging to the species glossina (*Glossina palpatis*) is very abundant; in places where this fly is not found no cases occur. Hence, it is highly probable that, as in the trypanosomiasis of the lower animals, the contagion is spread by a biting insect.

Symptoms.—The course of the disease is very insidious, as the trypanosomes may exist in the blood for a long time before entering and growing in the cerebrospinal fluid and causing the characteristic symptoms of sleeping sickness. Therefore, the symptoms may be divided into two stages. In the first stage there is only an irregular fever. In the second stage the fever becomes hectic, the pulse is constantly increased; there are neuralgic pains, partial œdemas and erythemas, trembling of the muscles, gradually increasing weakness, emaciation, and lethargy. The somnolence increases until a comatose condition is developed and death occurs. In the second stage trypanosomes are always found in the cerebrospinal fluid. Throughout the disease they are usually found in small numbers in the blood.

Duration.—The first stage may last for several years; the second, from four to eight months. The percentage of deaths in cases reaching the second stage is 100. Whether some in the first stage recover is not yet certain.

The trypanosoma Gambiense is irregularly pathogenic for some monkeys (*Macacus rhesus* and others), for dogs, cats, and rats. It is less pathogenic for mice, guinea-pigs, rabbits, and horses. Cattle and swine seem to be refractory.

Pathological Anatomy.—Congestion of the meninges; increased quantity of cerebrospinal fluid; hypertrophy of spleen, liver, and lymphatic ganglia.

Methods of Examination. BLOOD.—If the direct examination of the blood is negative, 10 c.c. should be withdrawn from a vein, and after adding a tenth of its volume of citrate of sodium it should be centrifuged for ten minutes, and the sediment examined in hanging drop and in smear.

CEREBROSPINAL FLUID.—Ten c.c. of the fluid withdrawn by lumbar puncture should be centrifuged for fifteen minutes and the deposit should be examined under 150 to 200 diameter magnification. Inoculation of susceptible animals should also be made with the blood or cerebrospinal fluid from the suspected case.

So far Musgrave and Clegg, after many careful examinations, have not found trypanosoma in any human beings in the Philippines.

Diagnosis of Trypanosomiasis in General.—This should be made as early as possible in order to prevent the spread of the disease. An early positive diagnosis can only be made by the determination of the blood infection. This is done in two ways: first, by the microscopic examination of a hanging drop of freshly drawn blood; second, by animal inoculation. In the microscopic examination it may be necessary to examine the blood of the suspected animal for several days in succession. The parasites are rarely absent in the early stages in domestic animals for more than a few days at a time, while in man the time may be much longer. If the trypanosomes cannot be found by this method, animal experiment should always be made. Monkeys, if possible, should be used, or if monkeys cannot be obtained, dogs or rats may be used. A few drops to 1 c.c. of the blood from the suspected animal should be inoculated intraperitoneally or subcutaneously.

Blood smears may be stained by any modification of the Romanowsky method.

Prophylaxis against Animal Trypanosomiasis.—The disease is readily controlled by preventive measures. There should be strict quarantine regulations governing the importation of animals. When the disease has once appeared the following general measures should be taken: 1. Suspected animals should be isolated. 2. All infected animals should be destroyed. 3. As far as possible all biting insects should be destroyed. 4. The bodies of infected animals should be protected from biting insects for at least twenty-four hours after death. 5. Susceptible animals should if possible be made immune.

Treatment.—Many drugs have been tried without success. Arsenic in various forms has been found to prolong life, but has produced no cures. It is, therefore, not to be recommended for the lower animals. Recently, Ehrlich and Shiga have found that a certain red product of the benzopurpurine series, to which they have given the name "trypan-

roth," has a preventive and curative effect in mice infected with *mal de caderas*. The curative effect is especially marked. As late as three days after infection with the trypanosome cures are effected. Inasmuch as the "trypanroth" is non-poisonous for the trypanosome *in vitro*, Ehrlich and Shiga suppose that a toxic substance is formed in the mice. The preventive effect of the trypanroth soon passes off, allowing the mice to become infected with trypanosomes two to three days after a preventive inoculation. On other trypanosomes and in other animals the results are not so good. Alternating arsenic and "trypanroth" may give better results.

Serum Therapy.—Various normal sera from different animals have been tried, with practically no success. A few have prolonged life. Thus Laveran and Mesnil state that human serum injected in sufficient quantities shows manifest action on the disease, and that sometimes cure results in mice and rats. Further, by alternating human serum with arsenic they obtained better results still. Kanthack, Durham, and Blandford showed that animals recovering from trypanosoma infection were immune to further infection. Rabinowitsch and Kempner have made a very careful study of immune serum produced by the trypanosoma Lewisi. Not only have they shown that an animal may be hyperimmunized and that then its serum in comparatively large doses inoculated into mice at the same time as the trypanosomes, or twenty-four hours before or after, allows no development of the organisms; but also Laveran and Mesnil state that the serum causes their rapid destruction by the leukocytes, though MacNeal, on the other hand, states that they are destroyed by a cytolytic action of the serum. This immune serum also has a similar action on the trypanosoma of Dourine. The serum of animals hyperimmunized against other varieties of trypanosoma is not as active as that obtained by the inoculation of trypanosoma Lewisi, *mal de caderas* giving the best results so far, but results that are not encouraging for practical treatment.

Koch suggested that an immunity might be established by the inoculation of attenuated parasites, and Novy and MacNeal have succeeded in attenuating cultures of trypanosoma Brucei, and have obtained some success in protecting experimental animals against virulent cutures.

Spirochæte Obermeieri (Spirillum of Relapsing Fever).

Until very recently this organism was classed with the bacteria, but it is now placed by Schaudinn and others with the flagellates, as it has many of the characteristics of the trypanosomes.

This spirochæte was first observed by Obermeier in 1873 in the blood of persons suffering from relapsing fever. It was found in large numbers during the height of the fever, it disappeared about the time of the crisis, and reappeared during the relapses. It was not found in other diseases. Obermeier considered it the cause of the disease, and his views were shown to be correct by the production of the disease in man and ape through experimental inoculation.

Morphology.—The organisms are long, slender, flexible, spiral or wavy filaments with pointed ends, from 16μ to 40μ in length and from one-quarter to one-third the thickness of the cholera spirillum (Fig. 148). They stain somewhat faintly with watery solutions of the basic aniline dyes, better with Loeffler's or Kühne's methylene-blue solutions, or with carbol fuchsin; best with the Romanowsky method or its modifications. They do not stain by Gram's method.

Biological Characters.—In fresh preparations from the blood the spirochætes exhibit active progressive movements accompanied by very rapid rotation in the long axis of the spiral filaments or by undulating movements. They are found only in the blood or blood organs, never in the secretions, and only during the fever, not in the intermissions, or at most singly at the beginning of or for a short time after an attack.

FIG. 148

Spirochæte Obermeieri blood smear. Fuchsin. × 1000 diam. (From Itzerott and Niemann.)

When kept in blood serum or a 0.6 per cent. solution of sodium chloride they continue to exhibit active movements for a considerable time. They may be preserved alive and active for many days in sealed tubes. They are killed quickly at 60° C., but they remain alive for some time at 0° C. Efforts to cultivate them in artificial culture media have thus far been unsuccessful, although Koch has observed an increase in the length of the spirilla and the formation of a tangled mass of filaments. But now with the cultivation of trypanosomes *in vitro* by Novy and MacNeal successful, one may hope for similar results with the spirochæte Obermeieri.

Pathogenesis.—In man, whether the disease is acquired naturally or by artificial inoculation, the organism causes the following symptoms: After a short period of incubation the temperature rises rapidly, remains high for five to seven days, and then returns to normal by crisis. About seven days later there is another sudden rise of temperature, but this time the crisis occurs sooner. A second or third relapse may occur. The organisms increase in numbers rapidly in the blood from the beginning of the fever, large numbers often being found in every microscopic field. They begin to disappear a short time before the crisis, and immediately after the crisis it is practically impossible to find them in the circulating blood. The mortality varies in different epidemics from 2 to 10 per cent. When monkeys are inoculated with human blood containing the spirilla they become sick about three and a half days later, but show only the initial febrile attack, or, at the most, an occasional short relapse. The organisms are found to have the same relation to the pyrexial period as in man. Blood from one animal taken during the fever induces a similar febrile paroxysm when inoculated into another animal.

Metchnikoff showed that during the intermissions when the spirilla disappeared from the circulating blood they accumulated in the spleen and were ingested in large numbers by certain phagocytes and finally were destroyed.

According to Lamb a certain amount of immunity is conferred upon monkeys (*Macacus radiatus*) soon after an attack, but it disappears quickly. If the serum is removed during this time it is found to have some protective action when mixed with the blood containing spirillæ, and also to cause agglutination of the organisms.

Infection probably occurs through the bite of blood-sucking insects.

Dutton showed (1905) that tick fever of the Congo, which is caused by an organism similar to that causing relapsing fever elsewhere, can be transferred to monkeys by the bites of young ticks at their first feed after hatching from infected parents. He accidentally demonstrated the fact that the disease can be inoculated into human beings through a cut surface, for after a wound received at autopsy he developed the disease which eventually caused his death.

Spirilla similar to the spirochæte Obermeieri have been found in birds.

Spirochæte Pallida Schaudinn in Syphilis.

The first two papers by Schaudinn and Hoffmann[1] appeared almost simultaneously. The paper in the former publication was illustrated with two photomicrographs, showing the form of the organism. These papers were quickly followed by two communications from Metchnikoff's[2] laboratory in the Pasteur Institute, confirming and accepting the discovery, and drawing attention to the interesting fact that Bordet and Gengou had observed the same micro-organism in a syphilitic chancre some three years before. However, as they failed to discover it in some syphilitic lesions which they subsequently studied they abandoned any future search for it. In this country the results of the above investigators have been corroborated by Flexner and Ewing.

The first publication of Hoffmann and Schaudinn dealt with a study of primary chancres, the enlarged glands of the groin attending these lesions, and flat condylomata in syphilitic patients. The study consisted in the examination of fresh specimens obtained from the surface and interior of the primary lesions and the interior of lymph glands and condylomata, and stained specimens from the same sources. Certain control examinations were also made of non-syphilitic lesions of the genitals and of mixed lesions of these parts. The results were quite uniform and suggestive. From the cases of simple syphilitic infection the lymph glands, condylomata, and interior of chancres showed a variable number of spiral micro-organisms of great tenuity, for which they propose tentatively the name spirochæte pallida, while the non-

[1] Arbeiten aus dem Kaiserlichen Gesundheitsamte, Berlin, April 10, 1905, xxii.; Zweite's Heft, 527; Deutsche medizinische Wochenschrift, May 4, 1905, xxxi. p. 711.

[2] Metchnikoff and Roux, Recherches microbiologiques sur sa syphilis, Bulletin de l'Académie de médecine, Paris, May 16, 1905.

specific lesions showed a second spiral micro-organism, for which they propose the name of spirochæte refringens (Fig. 149). The latter organism had, doubtless, been seen and described before by several observers. Schaudinn and Hoffmann did not find the first spirochæte in non-syphilitic lesions, nor did they find the second in the interior of the syphilitic lesions studied by them. From the study of Schaudinn and Hoffmann it is not difficult to explain the failure of previous investigators to perceive spirochæte pallida and especially the failure of Bordet and Gengou to obtain it in all of the several cases studied by them. The organism is difficult to see in the fresh state, and it is also highly refractory to staining, so that special methods are required to demonstrate it in fixed preparations. The description of the organism is as follows: In the length the spirochæte varies from 4μ to 10μ, the average being 7μ; in width the variation is from unmeasurable thinness to $\frac{1}{2}\mu$. The number of bends is from 3 to 12. The organism agrees in motility with the spirochætes rather than with the spirilla; there are three characteristic movements: rotation on the long axis, forward and backward motion, and bending of the entire body. There are indications of an undulating membrane, but none of flagella. The poles end in sharp points. No further details of structure have been made out thus far.

FIG. 149

The two spirochætes in the centre are Sp. pallida; the three others, Sp. refringens. (Schaudinn and Hoffmann.)

For the purpose of the study of the fresh material dilution with salt solution of the expressed juices of primary lesions, or the fluid drawn by aspiration from the lymph glands, is permissible. Prepared in this way the spirochætes were still actively motile, according to Schaudinn and Hoffmann, after six hours.

The staining is accomplished with difficulty, and the best results thus far have been obtained with Giemsa's eosin solution and azur. Schaudinn and Hoffmann recommend the following formula:

Twelve parts of Giemsa's eosin solution (2.5 c.c. 1 per cent. eosin, 500 c.c. water).

Three parts azur No. I (1:1000 solution in water).

Three parts azur No. II (0.8:1000 solution in water).

This mixture is to be freshly prepared. The films, which should be thinly spread, are dried in the air and then hardened in absolute alcohol for ten minutes, after which they are immersed in the stain from sixteen to twenty-four hours. They are to be washed in water, dried in the air, and examined in cedar oil.

Flexner and Noguchi have published a report upon the examination of four cases showing syphilitic lesions and two controls.

Case I. Male, aged twenty years. Luetic infection December, 1904. No regular treatment. He presented mucous patches of the tonsils and soft palate, and a fading rash of the trunk. Between the buttocks, flat condylomata. A condyloma was excised. Smears were made and stained in various anilines and with eosin-azur. Fresh preparations in salt solution were also studied. In the latter no characteristic organisms were found. The stained preparations were positive, showing a variable number of thin, lightly stained spiral organisms identified as the species described by Schaudinn and Hoffmann. The positive results were obtained with aniline-water-gentian violet and eosin-azur, the latter having given the most satisfactory results. The films varied greatly, even with the same method of preparation. In some the number of stained spirals was very small, while in others the number was large, a single field showing as many as five. In still other cover-glasses no organisms could be discovered. The spirals were long for the most part and showed from six to twelve bends or curves.

Case II. Male, aged thirty-four years. Burrowing non-syphilitic ulcer of the penis. Smears from the surface only could be obtained, and they were negative for spiral micro-organisms.

Case III. Male, aged twenty-three years. Mucous patches in mouth. Healed scar on penis. Enlargement and induration of glands of the groin. A small quantity of fluid, consisting of blood and lymph cells aspired from the enlarged glands. Fresh preparations negative; smears stained in eosin-azur showed a very few delicate, faintly-staining spiral organisms.

Case IV. Male, aged eighteen years. Infection in January. For several weeks active antisyphilitic treatment. Primary lesion on glans penis; marked swelling of the glands of the groin. Part of primary lesion was excised and fluid was aspirated from an enlarged gland. In the fresh fluid of the gland diluted in sterile salt solution several small and a single larger motile spiral organism were seen. None of the stained preparations from the primary lesion or the gland juice showed spirochætes.

Case V. Colored male, aged twenty years. No definite history of time of infection. Presented himself with phimosis and balanitis. After incision of the prepuce three separate elevated, indurated lesions regarded as chancres appeared. Moderate swelling of the glands of the groin. One of the lesions excised. Smears and fresh preparations were made from the base of the lesion. Fresh preparations negative. The smears stained in eosin-azur showed a moderate number of delicate spirals, agreeing with the description given by Schaudinn and Hoffmann. They varied in number in different cover-glasses, and in some could not be found. The curves numbered from eight to twelve.

Case VI. Male, aged twenty-three years. Non-syphilitic ulcer of penis; duration twelve days. Films stained in eosin-azur were negative for spirochætes.

Of the four cases of syphilitic lesions studied by Flexner and Noguchi the spiral organisms were obtained in stained preparations three times,

while in the two cases of non-syphilitic lesions studied they could not be found. An anomaly exists in respect to Case IV., in which the spirals were missed in stained preparations, while they appeared to be present in the fresh state. No explanation can now be offered for this occurrence.

Schaudinn and Hoffmann express themselves very guardedly regarding the significance of the spirochæte. They point out its presence in the typical lesions of the disease and its absence in the other forms of venereal disease studied. Important confirmatory contributions have come from Metchnikoff and Roux, who have demonstrated the same organism in the lesions of acquired syphilis in man and in experimental syphilis in the monkey and ape. In the last animals the material for study was obtained from the primary lesions produced by inoculation before ulceration had taken place. Additional confirmatory evidence of importance as regards the distribution of the spirochæte is supplied by the observation of Levaditi and Buschke and Fischer upon congenital syphilis. These writers found that organism in the pemphigus bullæ and papules of the skin, and, in cases coming to autopsy, in films from the spleen and liver. Schaudinn reports that he has obtained it also from splenic juice removed by aspiration from a syphilitic patient.

Metchnikoff and Roux draw attention to the irregularity of distribution of the organism as indicated by the variation in numbers upon the cover-slips. Others have observed the same irregularity, but it is not certainly established that the difference may not be due to the imperfect technique in staining. Metchnikoff and Roux and Levaditi prefer a more rapid method of staining the films, namely, that of Marino, which, up to the present, we have used but little. Should it serve as good a purpose as the slower eosin-azur, and should future study confirm the etiological position of the spirochæte, a rapid and useful and perhaps even a specific method of diagnosis would be afforded. Since the organism exists in the primary lesions and the glands of the groin in a demonstrable form, and since fluid from each can be obtained easily, with the infliction of little pain to the patient, and without in any way prejudicing the progress of his disease, we may look for a general study of the fluids obtained from these sources in suspicious and established forms of venereal disease with reference both to the occurrence and the specificity of spirochæte pallida.

CERCOMONAS.

Subclass: Flagellata.
Order: Protomonadina.
Genus: Cercomonas.

The members of this genus are round or oval flagellates with a long anterior flagellum and a more or less pointed posterior one which is sometimes amœboid. The vesicular nucleus is situated anteriorly and lying near it are one or two contractile vacuoles. Division into two daughter forms has been observed.

A number of cercomonada, none of them well studied, have been observed in different animals, as well as in man.

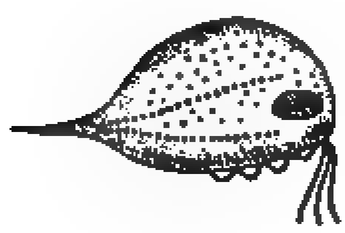
Cercomonas hominis (Davaine, 1854) was observed in the dejections of a cholera patient by Davaine. The body is 10μ to 12μ long and pear-shaped, pointed posteriorly. The flagellum is twice as long as the body. The nucleus is difficult to see. Davaine also reported a smaller form in the stools of a typhoid patient. Other observers have noticed similar forms in human stools, some associated with amœba coli. Similar forms have been seen also in an echinococcus cyst of the liver, in the sputum from a case of lung gangrene, in the exudate of a hydropneumothorax, and a few times in the urine (bodo urinarius). They are all probably harmless comensals.

Polymastigina.

The order polymastigina consists of flagellates having several flagella projecting from different parts of the body. The majority of the forms known are parasitic in certain fish.

Donné in 1837 described a form belonging to this group which he found in the human vagina, and which he therefore called trichomonas vaginalis. It has been found by other observers to be a frequent habitant

FIG. 150



Trichomonas vaginalis. (Blochmann.)

FIG. 151

Lamblia intestinalis. (Schewiakoff.)

of the vagina at all ages. It has also been found a few times in the acid urine of males. The mode of infection of the female is unknown. The body of the parasite at rest is pear-shaped, but during action its amœboid movements cause it to assume various shapes. The size varies from 12μ to 25μ long and 8μ to 15μ wide. The protoplasm is finely granular, excepting for two rows of larger granules which begin on either side of the nucleus and converge posteriorly. From the anterior part project three to four flagella, which seem to begin at a basal thickening near to or connected with the more or less oval, indistinctly vesicular nucleus. From the origin of the flagella an undulating membrane extends backward. The body also seems to possess a certain linear structure connected with the membrane. Contractile vacuoles have not been seen.

The trichomonas hominis Davaine, found frequently in the human alimentary canal, is very similar to the trichomonas vaginalis, but it is smaller and more pear-shaped. This form has been found often in acute diarrhœas, but no causal relation between it and the pathological process has been shown.

A similar form has been seen a few times in lung gangrene, aspiration pneumonia, and bronchiectasis.

Lamblia intestinalis (Lambl, 1859), a flagellate belonging to this group, parasitic in the small intestines of mice, rats, rabbits, dogs, cats, and sheep, has also been found occasionally in the human intestines. It is beet-shaped, bilaterally symmetrical, 10μ to 21μ long and 5μ to 12μ wide, possessing flagella 9μ to 14μ long. Anteriorly this species has a characteristic concavity, the rim of which seems to be contractile, forming a sucking apparatus. The eight flagella of the organism are arranged in pairs: one anteriorly, two laterally, and one posteriorly. The nucleus is situated anteriorly and has a central constriction. The protoplasm of the body is thick and hyaline. Contractile vacuoles have not been seen. Schaudinn has recently observed encystment, copulation, and complicated nuclear changes in this organism.

Infection follows the ingestion of the cysts with unclean food. The parasites fasten themselves to the free surfaces of the epithelial cells by their sucking apparatus, but seem to exert no harmful influence on their hosts. They have been found most frequently in poor children who play often in dirt containing the cysts. Repeated small doses of calomel will cause their disappearance from the feces.

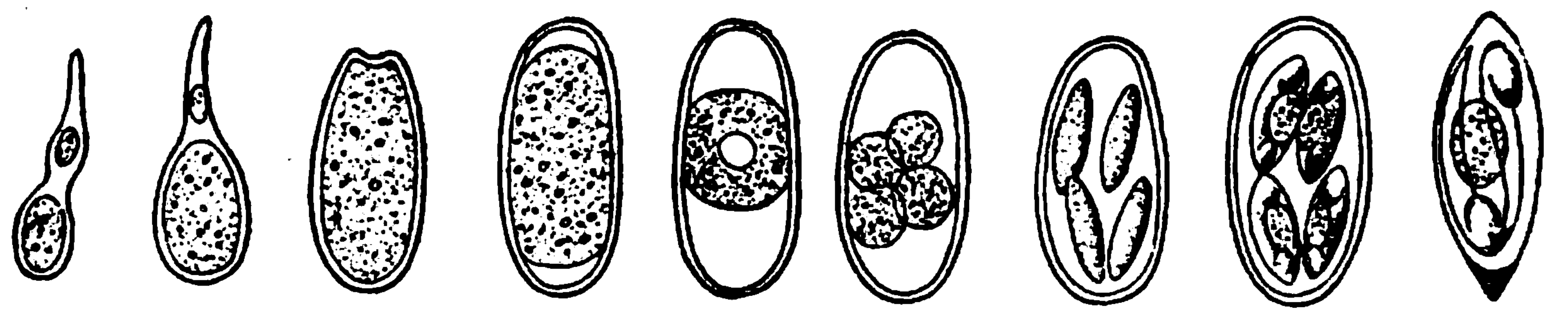
Coccidiomorpha.

Class: Sporozoa.
Subclass: Telosporidia.
Order: Coccidiomorpha.
Suborder: Coccidia.
Coccidium cuniculi (Rivolta, 1878).

The coccidium cuniculi is a frequent parasite of the rabbit. Young rabbits are especially susceptible, and extensive epidemics may occur in breeding houses. The symptoms are fever, diarrhœa, yellowish mucous discharge from the nose and mouth, and progressive wasting. The liver is much enlarged and shows throughout its substance variously sized gray-white tubercles, generally surrounded by a capsule, and containing a slimy mass of degenerated host cells, in which are embedded the parasites. The parasites are also found in the feces and in the epithelial cells of the intestines, gall-ducts, and liver. The acute stage of the disease lasts about three weeks. The contents of the coccidial tumors in animals that have withstood the infection may later be emptied, leaving only a mass of cicatricial tissue. In such animals the oöcysts may remain for a long time in the gall-bladder and intestines, and by passing out gradually with the feces may provide a source of infection for other animals. The infection is carried by food soiled with cyst-containing feces. The

cysts pass with the food into the stomach, where the cyst wall and the spore sack are destroyed and the sporozoites are set free. The motile sporozoites pass through the ductus choledochus into the liver, some probably passing into the intestines and infecting the cells directly, a later infection of the intestines occurring from forms developed in the liver. The organism develops within the epithelial cells of the liver and gall-ducts until the cells are finally broken down and tissue cysts are formed, within which, after more or less complicated changes, cysts of the parasite are again formed.

FIG. 152



Showing spore formation in coccidium cuniculi from the liver of the rabbit: *a* and *b*, young stage in the epithelial cells of the gall-ducts (the small oval is the cell nucleus); *c*, *d*, and *e*, the fertilized oöcyst; in *d* the protoplasm is beginning to shrink away from the cyst wall, and in *e* it has contracted into a spherical form; *f*, segmentation into four sporoblasts; *g*, elongation of the sporoblasts to form spores; *h*, four complete spores in the oöcyst; *i*, single spore more highly magnified, showing the two sporozoites and a small quantity of residual protoplasm. The life cycle has been fully worked out by Simon. (After Balbiani, from Doflein.)

A few cases of human infection of the liver with the coccidium cuniculi have been reported. The coccidium hominis Rivolta found a few times in the human intestines, as well as similar coccidia found in the intestines of lower animals, may belong to the same species.

Coccidium bigeminum (Stiles) is found in the feces of dogs, cats, polecats, and possibly human beings. The organism is characterized by the division of the oöcyst into two united cysts, containing four spores. The size is 8μ to 15μ. The life cycle is not well known.

Coccidioides Immitis Rixford and Gilchrist (1897).

The organism occurring in certain cases of skin and lung infection in man and described under the above name was classed by the authors with the coccidia; but it has been shown by Moffitt, Ash, and especially by Ophüls to be a mould, its fungous-like characters developing on the usual artificial culture media. The description given by Rixford and Gilchrist of the morphology of the parasite in the tissues is the same as that of the organisms studied by Ophüls. In each nodule formed in the disease one to several parasites are found either free or lodged in a giant cell. The parasites have the form of "rounded protoplasmic masses 20μ to 80μ in diameter, surrounded by a thick, enveloping membrane. Their multiplication is effected by a series of bipartitions which go on within the membrane; the latter then bursts and sets free the young parasitic elements, which grow *in situ* or are carried away by the blood or lymph." Among Ophüls' conclusions are the following: "The lesions produced by this fungus fall under the general head

of infectious granulomata, and consist partly in nodules resembling altogether those produced by the tubercle bacilli and partly in chronic abscesses. The adult forms of the parasite are more apt to produce nodules, the sporulating forms abscesses. The fungus is pathogenic for dogs, rabbits, and guinea-pigs, probably other animals also, and in them produces lesions very similar to those which we encounter in the human being in this disease."

CHAPTER XL.

MALARIAL PARASITOLOGY.

Suborder: Hæmosporidia.

THE causative agent of malaria is a protozoon which is classified with the sporozoa (suborder: hæmosporidia). It is now universally acknowledged to be the sole cause of what is properly included under the somewhat misleading term of malaria, a term which signifies "bad air."

The actual discoverer of the protozoon is Laveran, who recognized as early as 1880 the true nature of the dancing pigment which long before him had been observed in intermittent fever. The relationship of the paroxysm to the segmentation of the parasite was recognized later by Golgi. Recently the relationship of certain insects to malarial parasites has been demonstrated by Ross, and has been abundantly confirmed by many.

Before entering upon a description of these parasites in general, it may be stated that they have a double cycle—*i. e.*, as a tapeworm requires two hosts to complete its life cycle, so does the malarial protozoon. The intermediate host in this case is a mosquito. At this day there ought to be no more skepticism as to the role played by certain mosquitoes; but one must not forget that it is a special genus, and fortunately a relatively rare one, which causes the perpetuation of these protozoa. The characteristics of this mosquito are such that even people untrained in such observations can readily tell the malaria-carrying mosquito from its relatively harmless and prolific simile. Hence, a few useful points on the subject are given below.

The cycle in the human being is known as the asexual cycle, or the monogony; while the primary cycle carried on in the viscera of the insect is called the sexual cycle, or amphigony. There is undoubtedly in the human being a third unknown step, a sort of hibernation or lying dormant, attributable to a modification of a form which possibly belongs to the monogony.

In the asexual cycle the parasite grows and divides into segments which correspond to the young form from which the parasite originates without the influence of a sexual element. This process of apparently agamogenetic division goes on periodically for a limited length of time. It seems as if the power for self-reproduction is exhausted after a time, and as if possibly also the blood of the host has formed circulating compounds antagonistic to the parasite. These explanations for the time limit seem reasonable.

At all events the sporulation ceases after a while, and there are distinct changes in the morphology of the parasite. Finally, certain bodies are formed which do not segment, and it is these which will now be described in detail. They are known as gametes, or sexual forms, and represent a male and female element which, however, cannot conjugate in the human blood. When certain species of mosquitoes imbibe blood containing such gametes, the process of conjugation is carried on in the chyme stomach of the insect. How this proceeds will be described presently; suffice it to say here that cysts are formed in the stomach wall of the insect, which when matured discharge an enormous number of filamentous sporozoites into the body cavity of the mosquito, whence they reach what is usually called the salivary gland of the insect. From this gland it is but a step to the proboscis, and when the insect thus infected "bites" a human being numerous sporozoites pass into the circulation. Just how these sporozoites are transformed into the well-known young form seen on the red corpuscle is not known.

While these processes are rather fully understood, the relapses of supposedly cured cases are shrouded in mystery. The observations tend to show that there is a difference in the parasites of relapses when compared with those of a recent primary infection, and for my part I do not usually have any difficulty in telling the parasites of relapse from those of a recent primary infection. Moreover, I have also found that the parasites of a supposed second infection resemble those of a relapse, and in the light of this observation I doubt if a second infection in the proper sense of the word is ever possible within a certain length of time. It is not within the scope of this article to give a detailed description of the forms which in my opinion point to a relapse; suffice it to say that the parasites in question show a tendency to remain dwarfed, and show fewer chromatin bodies and greater irregularities in division than those of what I term a recent primary infection. These abnormalities may be referable to the persistent effect of antibodies. One form of parasite is so constant in protracted cases and differs so much from the ordinary gamete that a special description will be given.

The different varieties of malarial and kindred parasites in man and animals have, as might be expected, much in common. Hence a general description of the cycle of one form will suffice to elucidate the cycle of the others.

Generalities of Cycle.—A young, amœboid, colorless, more or less rounded parasite, measuring approximately two microns in diameter, is seen attached to a red corpuscle. After a certain length of time, usually a few hours later, granules or rodlets of pigment make their appearance; these granules represent the hæmoglobin on which the parasite has been feeding, and it is now transformed into what is commonly termed melanin. They will be seen to be in passive motion, the degree of motility depending upon the currents in the protoplasm in which they are embedded, and they vary in the different varieties and at different stages of the asexual phase. After a further lapse of time this pigment is seen to gather centrally and evidences of cell division

can then be made out; there may be a distinct rosette-like arrangement or not. The next step is the breaking up of the parasite into segments free from pigment. The further observation of these segments, each of which represents a young parasite, identical with the young form from which its progenitor was built up, is a matter of difficulty. I had the good fortune of watching one in a fresh-blood preparation from the moment of its detachment from the mother substance, and saw it approach corpuscle after corpuscle. Its movements were gregarine-like. It did not attach itself to a corpuscle within an hour, presumably because of the change in the nature of the blood and its plasma.

In the blood stream it is but rarely seen free in the plasma, and it would appear that it rapidly attaches itself to a neighboring corpuscle. That would tend to explain the fact that in certain forms of malaria it is not an uncommon thing to find several parasites in one corpuscle; this is seen especially in those cases where segmentation takes place in the bone-marrow, and where on account of the absence of the velocity of the blood stream the parasites are less apt to be scattered than in the circulating blood.

It is to be remembered that the process of growth and division cannot be readily observed in all the forms of malaria because certain parasites complete their asexual cycle in the viscera, chiefly also, it seems, in the bone-marrow and spleen.

Three types of parasites are recognized. Classified zoölogically they are: Plasmodium vivax (tertian), malariæ (quartan), and præcox (æstivo-autumnal).

The life cycle of each presents certain characteristics which hold it apart from the others. Some authors suggest a unity of parasites, but the mere fact that the gametocytes of tertian and æstivo-autumnal fever differ absolutely from each other is a capital reason why this is improbable; there are many other striking differences.

A brief consideration of the salient facts follows:

Plasmodium Vivax, the Parasite of Tertian Ague.

The young form is more difficult to find and to recognize than any of the other forms. A pale area is seen on an otherwise unaltered red corpuscle, situated usually eccentrically, about one-tenth the size of the red corpuscle or about one-fourth its diameter, when at rest presenting a rounded appearance, usually actively amœboid, throwing out pseudopodia which are distinct, never remaining long in the same focal plane, frequently dipping, so to speak, into the substance of the corpuscle. It is often called the hyaline form because it is free from pigment, but it is not hyaline in the proper sense of the term. It is also called the ring form, because of its resemblance to a ring in stained preparations; but it is never a true ring.

The forms intermediate between this and the segmentation stage are simply larger parasites, which are readily found on account of the pigment granules which they contain.

In the earlier stages they resemble the younger form except that the pseudopodia appear to be exaggerated; besides there is a rapid dancing of the pigment due to protoplasmic currents in the parasite. The infected corpuscle is swollen and paler. Later the parasite occupies the greater portion of the corpuscle, which is now more difficult to make out. The pigment is still more evident, so that this form is therefore most readily found.

The anatomical changes which have been going on in the parasite are best studied in properly stained smear preparations. They become presently sufficiently distinct to be followed in the living parasite; the pigment gathers more or less centrally into a compact mass, and a peripheral notching indicates that the parasite is preparing to divide into a number of segments; the number of segments varies even in perfectly typical acute cases, but from twelve to twenty may be counted as soon as the parasite has become fully mature. Suddenly these spheroidal segments separate from each other; a corpuscular remnant and the pigment float away and are ultimately carried to the spleen. Phagocytic cells ingest these pigment masses. The young parasites attach themselves to red corpuscles as already stated. The mature tertian parasite is usually larger than a red corpuscle.

The varying number of segments is possibly accounted for by the difference in size, age, and constitution of the infested corpuscles. Later, when the blood may have formed antibodies, irregularities are still more readily explained. If suitable smears are made from blood containing such tertian organisms the life cycle can be studied much more closely.

The making of these smears is a simple matter. There are the cover-glass and the slide methods, both of which have their peculiar advantages. To make a cover-glass preparation, two square, very thin, hence flexible, cover-glasses are cleaned. Holding one with thumb and index fingers by opposite corners the tip of a drop of blood obtained by needle puncture of finger or lobe of ear is made to touch the centre of the cover-glass, and the second clean cover-glass held similarly is allowed to fall upon the first one in such a manner that the corners do not coincide. The blood droplet spreads by capillarity into a thin film, which is a sign to pull the two covers apart in the plane in which they lie; good results depend upon cleanliness, rapidity, and success in sliding the two covers apart.

A simpler way is to polish two slides. The tip of the exuded blood drop is made to touch one slide near one end and the edge of the second slide held at an acute angle to the first one is made to bisect the drop, which will spread at the point of contact by capillarity across the slide. Upon pulling the second or spreading slide over the first slide, never changing the angle and applying gentle pressure, a thin layer of blood suitable for examination will be formed. A slide made in this manner should be dried immediately by agitation in the air. It may then be fixed and stained in various ways. I would recommend a modification of a method devised by me in 1900, which is given here below, together with some others.

In a suitably stained preparation (using a chromatin dye) the young parasite appears to be a disk consisting of a basic (blue) periphery, the body, including a metachromatically stained, rounded, compact (red) chromatin body, often called the nucleus, which tends to give the parasite the form of a signet ring, and of a central, pale, unstained area, known as the achromatic zone.

Later stages up to a certain number of hours show simply changes in size and outline of the blue-stained body. Then the red chromatin body loosens up and presently its substance divides into an increasing number of angular pieces, which are the first evidence of regeneration. By the time that chromatin division is completed the angular chromatin masses will have assumed a rounded form, and will be seen to exhibit ultimately the same strong affinity for certain dyes which is seen in the compact chromatin body of the young ring-like form. At this stage the heretofore scattered pigment appears in one clump. Good technique will always show a corpuscular remnant even at this time. The achromatic zone mentioned will be seen to develop with the chromatin, and when the next step, namely, the division of the body of the parasite, is seen to be completed, there will be as many achromatic bodies as there are chromatin bodies, each having an equal share of the basic mother-body, each representing a young parasite. These young parasites next escape from their envelope or whatever substance may have held them together, are set free in the plasma, and attack without delay the corpuscles.

It is an open question as to whether the parasite sits *on* or lives *in* the corpuscles. The latter supposition appears to be more plausible, especially because the segmenting form retains to the last a corpuscular remnant and because the young amœboid form may be seen to dip into the corpuscle, leaving one focal plane and appearing in a deeper one. How could that be possible if it simply adhered to the surface? The

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DESCRIPTION OF PLATE II.

(The numbers are placed immediately above the respective form.)

1 to 4. Young forms; unpigmented.

5 to 8. Gradual pigmentation and growth of the parasite; the chromatin is loosening up.

9 to 13. Active division of chromatin from two into twelve or more pieces, which are at first angular, but become rounded.

14 to 15. Complete segmentation; collection of pigment in single lump.

16. Bursting of segmenting parasite and liberation of young forms, each of which *de novo* infects and destroys a new corpuscle.

17 to 19. Young male sexual forms.

20. Male sexual forms (microgametocyte). The coiled-up, centrally situated chromatin fibrils are the flagella or microgametes.

21 to 22. Female sexual form; stain deeper than in male; less chromatin, situated peripherally; often extracorpuscular; macrogamete. (These gametes are the analogues of the crescentic bodies of æstivo-autumnal malaria.)

23. Abnormally situated accessory chromatin body.

24. Two parasites maturing side by side.

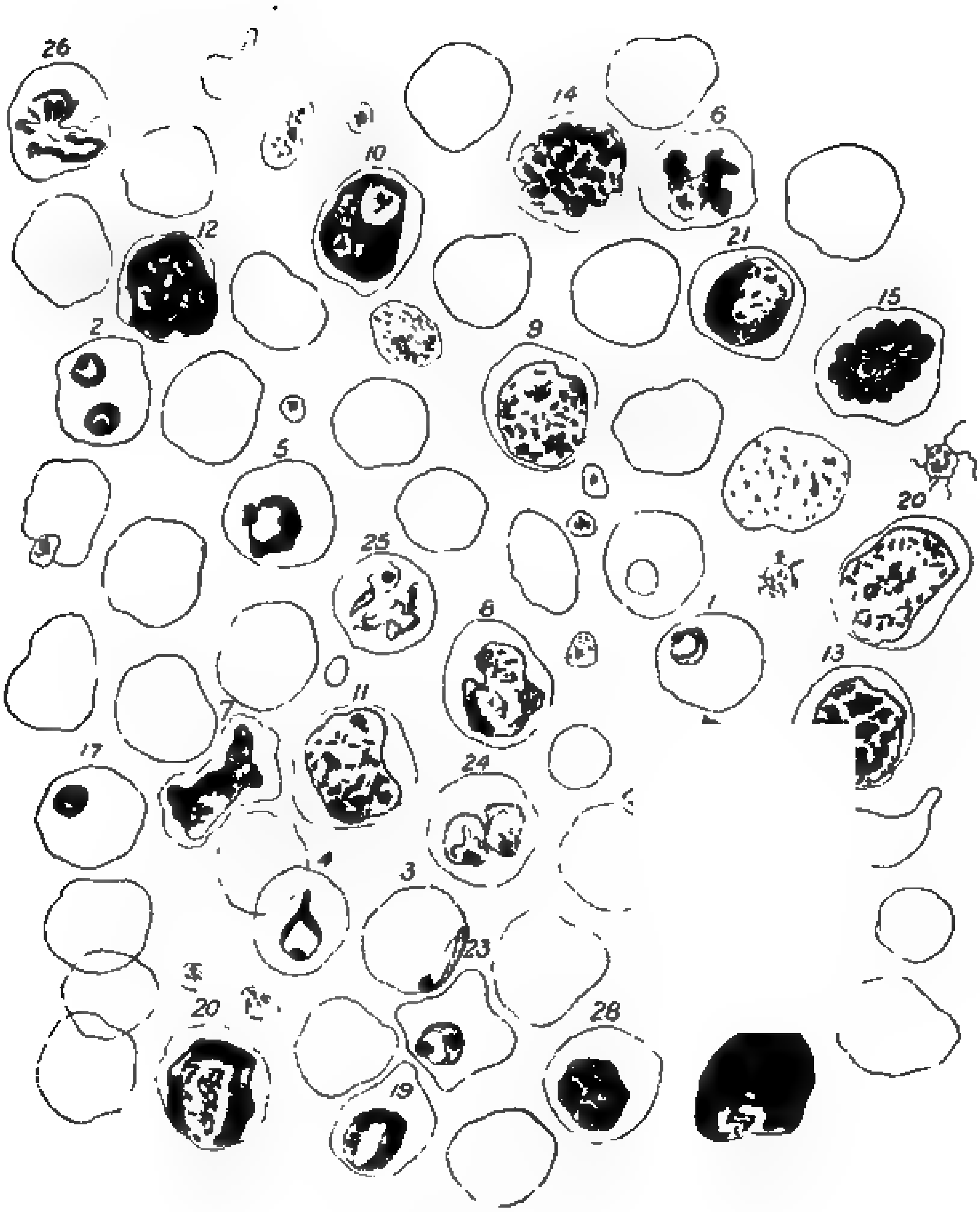
25 to 26. Effect of quinine on body of parasite.

27. Resistant form capable of causing relapse (?).

28. Abnormal segmentation of immature parasite.

The various oval and rounded bodies are blood platelets. The red blood corpuscles are simply outlined; their color and degree of degeneration when infected will depend entirely upon the technique. The body of the parasite is blue; the chromatin body is carmine; the pigment is simply shown in black.

PLATE II.



Various Stages in the Development of the Malarial Parasite.
Tertian type. (Goldhorn.)

denser protoplasmic boundary zone of the corpuscle offers no resistance to the parasite.

The technique recommended below shows that the infested corpuscle early undergoes a granular degeneration, which, curiously enough, in the first few hours resembles the ordinary granular stroma degeneration with basic affinity, while it is later seen that the affinity of the then more numerous granules is more acid or at least the staining is no longer orthochromatic, the blue being superimposed by a red; in other words, these granules stain later metachromatically. The greater the loss or transformation of the hæmoglobin the greater the number of granules. This holds good only for tertian parasites, the æstivo-autumnal variety causing practically no appreciable change though the same technique be used.

But the mere recognition of the malarial parasite in the blood is insufficient to-day. Not only should the physician know the variety with which he is dealing, but also it will be to his advantage to know something about the progress which the disease is making by study of the circulating parasite. This will seem difficult at first, but a relatively small amount of study will show the value of the recognition of certain changes.

In tertian fever much can be learned from the study of, let us say, three typical cases, viz.: (1) a recent primary infection; (2) a primary infection, but of long standing; (3) a relapse. The student who knows the forms occurring in the typical recent primary infection will readily observe many morphological differences between these and the parasite of the protracted case. He will then also find at least two forms which are not readily classified with those of his recent primary infection. These forms are the gametocytes or gametes, the analogues of the crescent.

The microgametocyte of tertian malaria is a large parasite with little affinity for methylene blue, carrying more conspicuous pigment arranged frequently as a wreath around a large achromatic zone in which filaments of chromatin lie. These filaments are the microgametes, also known as the flagella. When living the pigment of this parasite is immotile until just before the parasite flagellates, at which time an unusual activity of the granules is seen.

The second form is the macrogamete, which is an extracorpuscular body, staining deeply in methylene blue, having a hazy chromatin mass in indistinct achromatic zone usually situated peripherally.

The young form which grows ultimately into a microgametocyte is ring-shaped, with a heavy chromatin body well within the achromatic zone.

In relapses a third form is met with which has a compact chromatin body, surrounded by a paler area, that can, however, be stained pinkish by chromatin dyes. The recognition of these forms is of the utmost importance because the prognosis depends upon them. They are quite resistant to quinine and other drugs, and it appears as if cases in which these forms are seen are much more prone to relapse than promptly treated recent primary infections.

Also the absence of gametocytes from the blood means that no

amount of such blood that anopheles mosquitoes may imbibe can ever infect the mosquito.

When studying old cases and relapses it will be seen that the characteristics of the three varieties are lost to a certain extent. But other factors will have changed accordingly; the paroxysms will have become irregular, protracted, less severe, and will eventually occur widely apart. Now, as the paroxysm depends upon the segmentation of the protozoon or the probable liberation of a toxin, any changes in the morphology might be expected to influence the periodicity and character of the paroxysm, and such is actually seen to be the case.

An abnormal temperature chart can usually be explained by proper study of the forms of parasites present. If quinine has been administered it is to be considered, as its effect on the morphology of the parasite is profound and rapid. The part most and first affected is the blue staining body; later follow eccentricities of the chromatin, such as multiple bodies in young forms, extension of chromatin bodies, and dwarfing, just such changes as might have occurred in time if the body had been allowed to combat the parasite without the aid of drugs.

The notion that the parasites can be found only at the time of the paroxysm is still in the minds of many; it is erroneous.

The Æstivo-autumnal Parasite.

In a recent primary infection only young forms can be found in the peripheral blood; their pigment is so scanty as to render their recognition in fresh blood a matter of experience; dancing pigment is practically never seen in such cases.

Later in the disease the gametocytes develop; they are absolutely characteristic, readily found, and of diagnostic value.

In the fresh blood the young parasite is seen as a fairly sharply defined, rounded, slightly amœboid body; the infected corpuscle may be crenated; it may have what has been termed a "brassy" appearance.

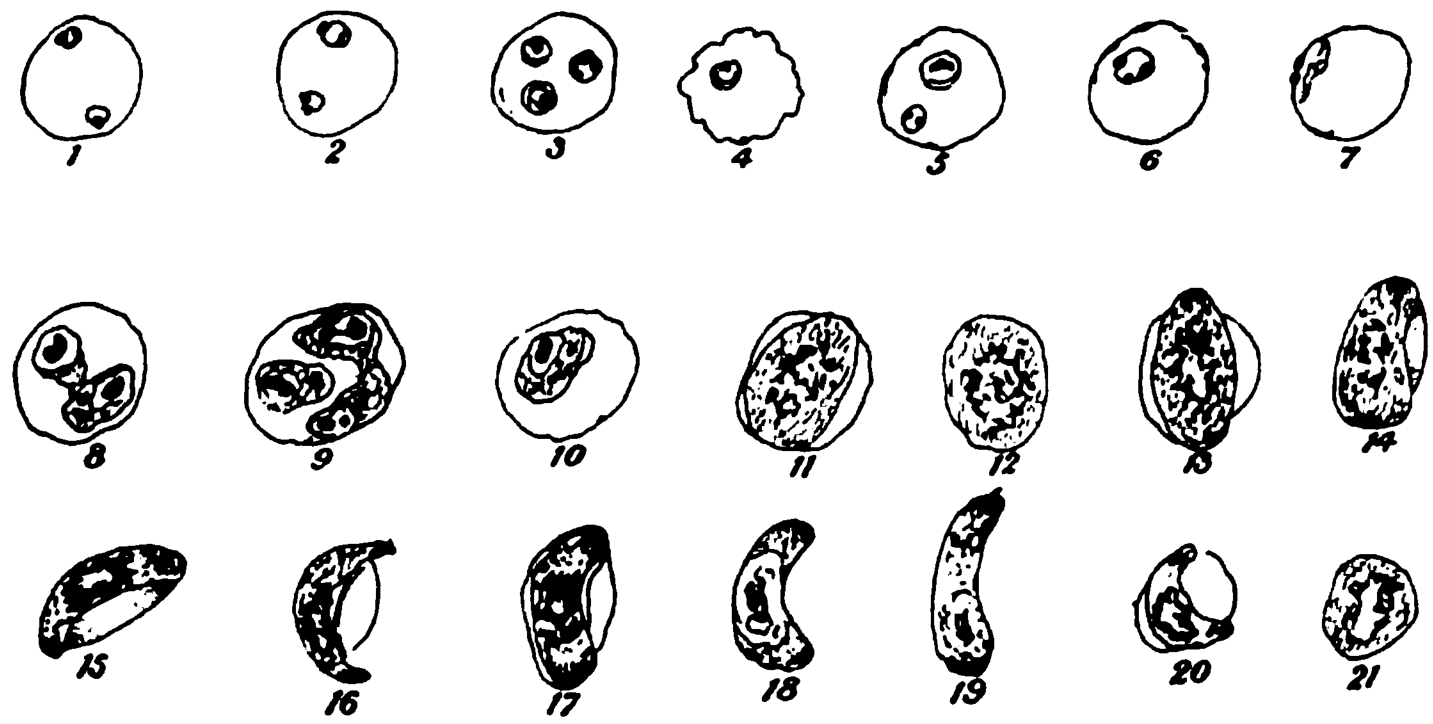
Such forms stained show extremely thin and small rings, with one or more chromatin bodies, situated not infrequently within the achromatic zone. There are frequently several parasites in one corpuscle; five, six, and seven have been seen. Later the parasite shows a few heavy, blackish, pigment granules, and an increase in the size of the body is seen. It is very exceptionally that one finds in the peripheral blood forms approaching segmentation or actually segmenting. The various steps of chromatin division seem to take place chiefly in the bone-marrow and the viscera. The parasite does not ordinarily attain the size of the tertian form, and there are usually fewer segments. Their arrangement is apt to be very symmetrical.

Upon developing the gametocytes these are seen at times in large numbers. They are first ovoidal and later crescentic. The blackish pigment lies in a wreath around the thus hidden chromatin filaments. There may be a corpuscular remnant or not. There seems to be a sort

of capsule at times. The ends stain more deeply in methylene blue than the rest of the parasite, a phenomenon which is known as polar staining.

The pigment is quiescent in the fresh specimen. Upon prolonged observation, say twenty minutes or less, according to temperature, etc., these crescentic bodies are transformed into spherical bodies; the pigment of certain ones of these becomes actively motile, due to internal agitation of the chromatin fibrils, which will presently emerge as flagella. Their movements are very rapid, corpuscles are knocked about, and finally these flagella, which represent the male sexual element, the spermatozoid, so to speak, become detached and go in search of the female element. In birds one may actually observe the process of conjugation in slide preparations even without the aid of a moist chamber and heat. This transformation of crescentic bodies never occurs in the human blood. It will be seen that it belongs to the sexual cycle which occurs in the stomach of the mosquito.

FIG. 153



Æstivo-autumnal type: 1 to 7, various young non-pigmented forms; 8 to 10, larger forms from peripheral blood; 11 to 14, various ovoid bodies; 15 to 17, crescentic bodies; 18 to 19, crescentic bodies after removal of pigment showing chromatin filaments in achromatic zone; 20 to 21, abnormal forms.

Crescents do not, in my opinion, ever divide, either agamogenetically or otherwise, in the blood. Forms which show a constriction are rather to be referred to a twin maturation. Nor do I believe that the chromatin of microgametocytes ever divides by segmentation; it seems to me that the coiled-up filaments are rapidly rearranged and then individually extended as so many flagella or microgametes.

In fatal cases the formation of crescents may not take place; the blood infection with young parasites is then enormous, every field of the microscope showing numbers of them.

In the study of æstivo-autumnal fever it is to be remembered that crescents when found indicate that the disease is of some standing, for such sexual forms (gametes) are not formed until the asexual propagation is waning. The recognition of these ovoidal and crescentic bodies is easy. But as there are no readily discoverable pigmented forms in

the peripheral blood in the early stages, it is necessary to be thoroughly familiar with the young æstivo-autumnal forms. Chromatin staining for them cannot be too much recommended, as there is little that is characteristic about them when they have been stained with methylene blue alone. Many a serious error has been made by adhering to the antiquated idea that parasites should be looked for in the fresh blood, as these young, non-pigmented, so-called hyaline forms cannot be readily recognized by the inexperienced, while it is an easy matter to know and classify them when properly stained.

The Quartan Parasite.

The recognition of the quartan parasite in its early stages in the fresh blood is not as difficult as that of the tertian form, but in stained preparations it is often indistinguishable from the latter. The living amœboid young form is more refractive than the young living tertian form, more like the æstivo-autumnal form, and it is just as sluggish in its movements. Here, too, the corpuscle is often shrunken and looks as if it contained more hæmoglobin than in the case of infection with the tertian parasite.

The growing parasite shows fewer pigment granules than the corresponding tertian one, and it is apt to form a band across the infected corpuscle. Segments are few in number, as a rule, and the parasite remains

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DESCRIPTION OF PLATE III.

1. Typical young tertian form; the corpuscle shows incipient degeneration; corpuscle to left above shows a blood platelet.

2. Abnormal young form, showing small accessory chromatin body.

3. Two parasites; one a normal young form; the second large form in crenated corpuscle is an unusual abnormal form with very large achromatic area.

4, 5, 6. Æstivo-autumnal parasites; single, double, and triple infection; central elongated chromatin bodies. These forms are about the largest usually seen in the peripheral blood; no degeneration of corpuscle.

7. Tertian parasite, about ten hours old; marked degeneration of corpuscle.

8. Double infection of a corpuscle in tertian fever; marked degeneration of corpuscle.

9, 10, 11. Large tertian parasites showing division of chromatin previous to segmentation.

12 and 14. Complete segmentation of tertian parasite.

13. Double infection of corpuscle, one parasite reaching maturity, but showing unusually small segments; the second one atrophied.

15. Tertian parasite, old case; while the parasite is only half-grown, the chromatin has split into several compact masses. Degeneration of infected corpuscle.

16. Dwarfed tertian parasite, smaller than a red corpuscle, but showing five compact chromatin bodies; resemblance to quartan rosette.

17. Microgametocyte of tertian malaria; prominence of blackish pigment surrounding a large achromatic zone in which the microgametes lie coiled up.

18. Tertian macrogametes.

19 to 23. Crescentic bodies of æstivo-autumnal malaria.

19. Typical gamete; pigment surrounding achromatic area; no chromatin shown; the "bib" is present. (Male?)

20. Semiovoid gamete. (Female?)

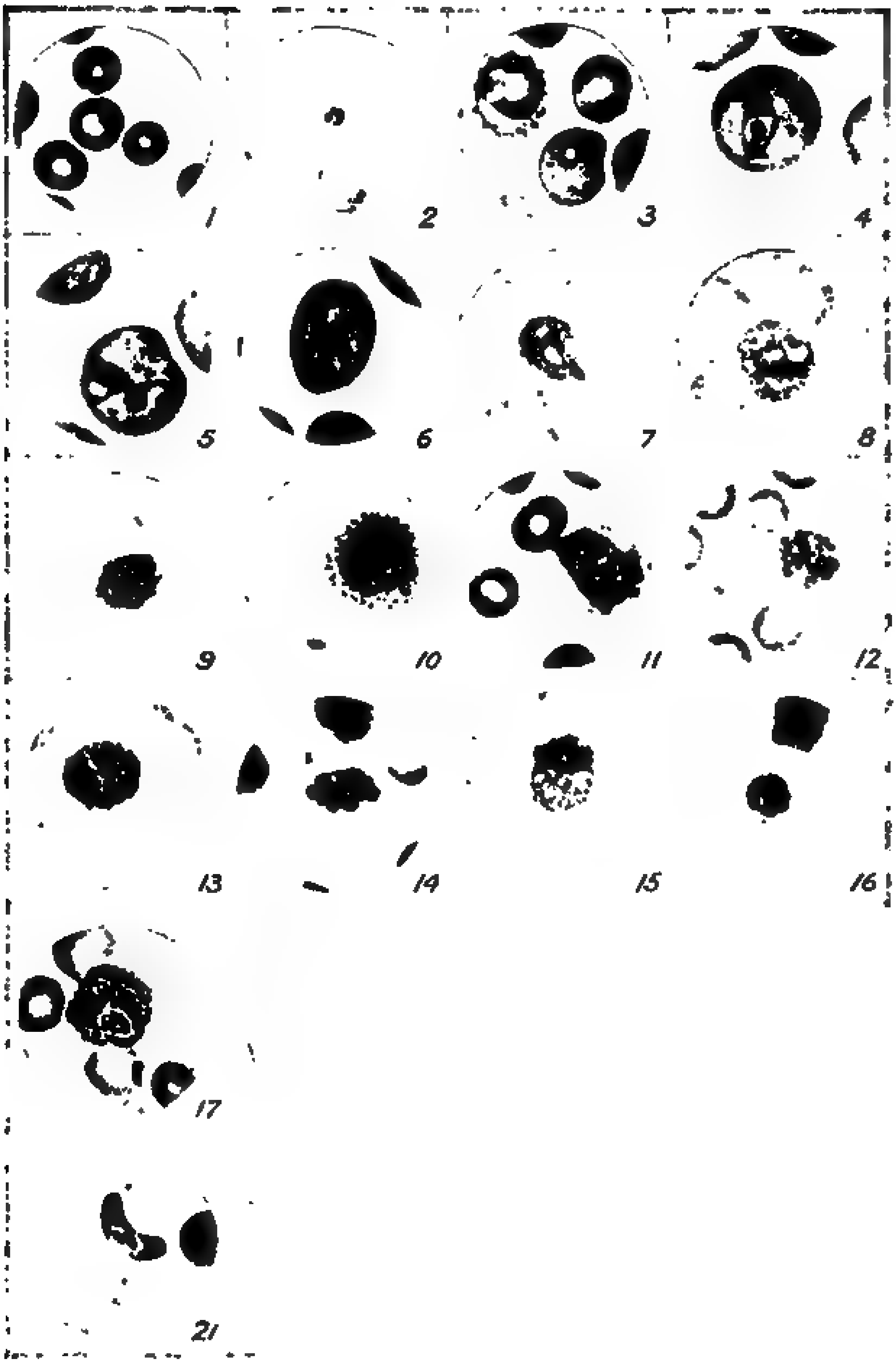
21. Pigment removed. Elliptical achromatic area in which the microgametes are seen.

22 and 23. Pigment removed; chromatin more compact; possibly female elements.

24. From a case of pernicious malaria with rich infection; only hyaline forms in peripheral blood. Below a large blood-platelet.

NOTE.—As the amplification is not uniform a comparison of the parasites with the blood corpuscles shown should be made in order to have a correct conception of their size.

PLATE III.



Photographs of Tertian and Æstivo-autumnal Malarial Parasites in Different Stages of Development. (Goldhorn.)

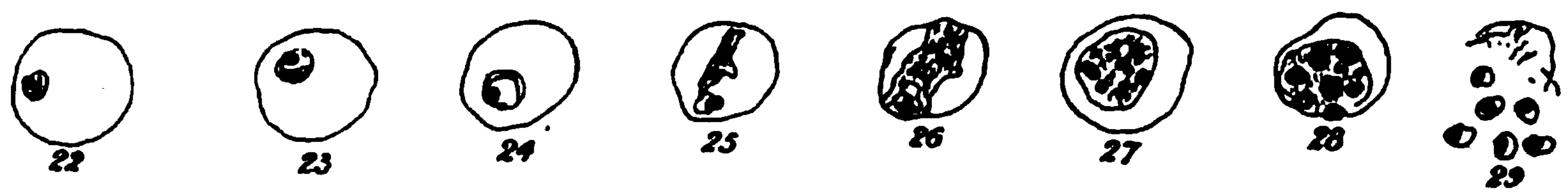
dwarfed. It never attains the size of the tertian form. All the steps leading to completed segmentation are seen in the peripheral blood.

The gametes are not well known. Cases of quartan are relatively rare.

General Observations.—It should be remembered that there is no quotidian form in this country. Quotidian paroxysms are either a double tertian or a triple quartan infection.

It is also interesting to note that the fever curve becomes atypical even in those cases in which no quinine has been given. The excursions will be less, but the temperature will stay up longer and drop more gradually; or there may be two consecutive paroxysms usually varying in intensity. The parasitic forms in the blood will vary accordingly.

Fig. 154



Quartan type: 22 to 24, young forms; 25 to 26, tendency to band formation; 27 to 29, segmentation. (All forms drawn from stained smears.)

The relationship between segmentation and paroxysm is always noted in tertian cases, and it is reasonable to suppose that the occurrence of the paroxysm is referable entirely to the liberation of toxic substances resulting from metabolic activity of the parasite within the corpuscle. That there should be a toxic product seems highly probable, and its amount must be considerable in heavy infections. Cases showing an infection of 1 to 5 per cent. of all corpuscles are not infrequent; the destruction of from 50,000 to 200,000 or more corpuscles per cubic millimetre of blood leads to the rapid deglobularization of the blood; hence the deficiency in numbers; add to this the effects of the metabolic products, and little is left to the imagination to explain the pronounced anæmia. Furthermore, the corpuscular remnants will be largely carried to the spleen; hence its hyperplastic condition and pigmentation.

Malarial parasites can always readily be found in recent primary infections, and it is usually only in old cases that the search becomes difficult; one is, however, generally rewarded by finding them if one looks long enough for them.

A helpful sign is the finding of pigment in mononuclear leukocytes, which are seen about the time of a chill, or to the period symptomatically corresponding to it.

Free pigment cannot be used as a means of diagnosis, as it may be impossible to tell it from dirt or dust.

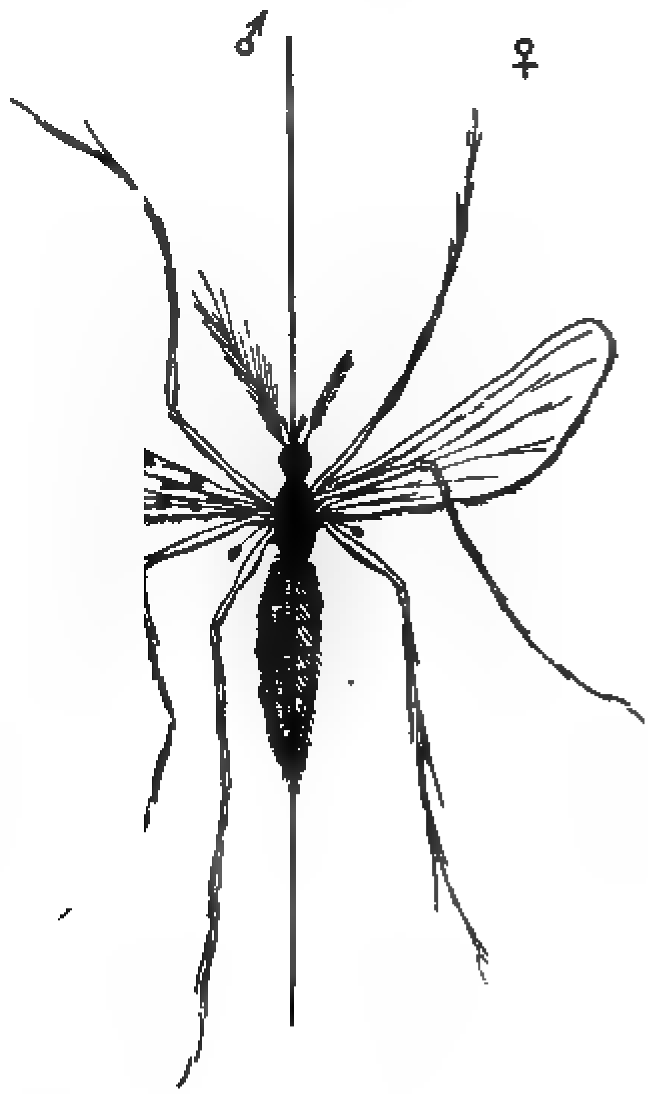
A small dose of quinine may drive all parasites of the monogony out of the peripheral circulation; at all events, the finding of them becomes, in the absence of gametocytes, a matter of time and experience, especially also as they may be much altered in appearance.

Immunity from malaria appears to exist as natural and acquired immunity. Whether the usual assertion that contraction of disease by pyogenic micro-organisms depends upon a lowering of the resist-

ance of the body holds good in the case of these protozoa is uncertain. Studies made by me on over fifty birds infected with proteosoma showed that (1) young birds invariably became infected, while old ones rarely did; (2) objective symptoms in young birds were much more pronounced than in previously healthy older birds; (3) old birds whose resistance appeared to be lowered by disease were readily infected; (4) reinfection of all birds appeared to be negative.

The Malaria-carrying Mosquito (Anopheles).—Only generalities can here be considered. The common mosquito, often day-flying, belongs to the culicidæ; it cannot carry human malaria. It is distinguished

Fig. 156



Culex. Anopheles. Anopheles. Culex.

(From Doflein.)

from its night-flying or dusk-flying relative by its assuming a different posture on the perpendicular wall. While the culex holds the body more or less parallel with the surface, the body of the anopheles stands off at a marked angle.

Wings of culicidæ are unspotted; those of *Anopheles maculipennis* and *Anopheles punctipennis* are spotted.

The proboscis of anopheles points toward the resting surface, while that of culex does not do so.

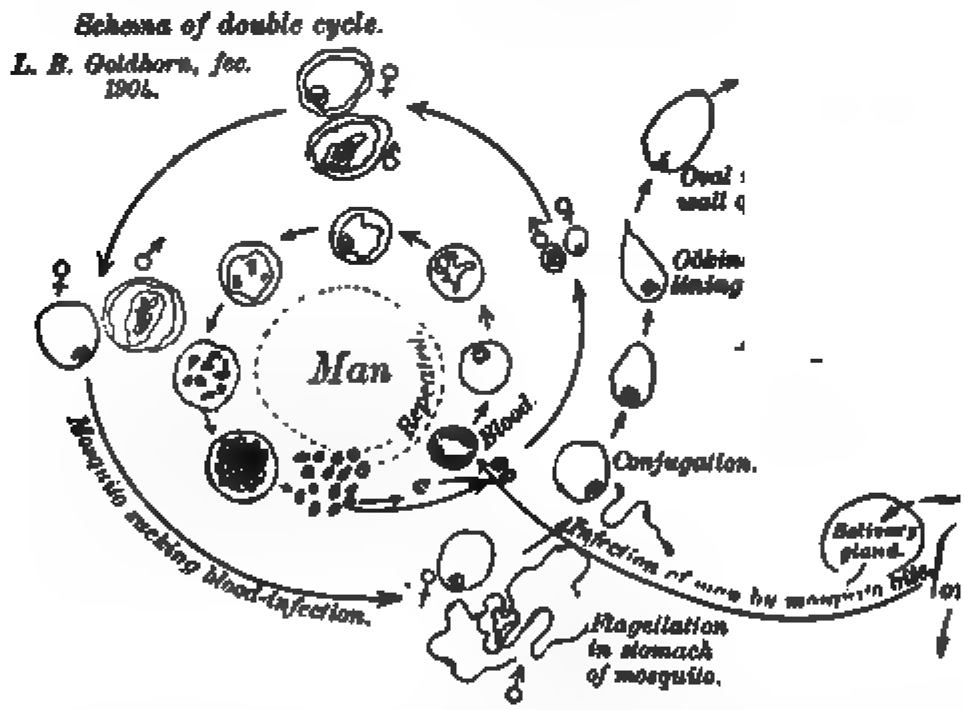
Anopheles varieties bite usually in the early evening, while those of culex bite almost at any hour.

The male mosquito is readily told from the female by its plumed antennæ, those of the female being inconspicuous.

The Cycle in the Mosquito.—If an ordinary mosquito (culex) is allowed to imbibe the blood of a malarial patient whose blood shows gametocytes there will be simply a digestion of such blood in the mosquito, but no anatomical changes. If, however, an anopheles mosquito ingests such blood, immediate changes follow. It should be remembered that only female mosquitoes bite; hence, they alone can be responsible for the spreading of the disease.

The flagellation of the male parasite described above will promptly take place; the free flagella conjugate with the female element in a manner comparable to the impregnation of the ovum of higher animals by spermatozoids.

FIG. 156



Inner circle-asexual reproduction; moist-chamber observation shows no flagellation. Outer circle-formation of (sexual) gametes; moist-chamber observation shows flagellation.

Infection of man through gastro-intestinal and respiratory tract, the infected mosquito dying in water, drying in air or sucking plant-juices, infecting fresh vegetables (theoretical)

This product of conjugation remains for a number of hours in the juices of the chyme stomach of the mosquito, changing gradually from a spherical, immotile body into an elongated wormlet endowed with motility. This penetrates the epithelial lining of the stomach and rests in the tissues; here it changes into an oval, then into a round body, which grows in the course of the next few days enormously, forming a cyst which projects into the body cavity. Meanwhile the chromatin will have been very active. It will have divided into numerous nuclei, which become arranged around inactive portions, and filamentous sporozoites develop from this chromatin. These sporozoites ultimately fill the cysts, which rupture, setting them free into the cavity of the mosquito's body; they then make their way to a glandular structure in the thoracic cavity of the insect, the so-called salivary gland, which in turn is in immediate connection with the biting and sucking apparatus. If now such an infected mosquito "bites" a human being the lubri-

cating fluid of the puncturing apparatus will carry sporozoites into the blood. The stages of development in the mosquito require about seven days.

Staining Methods. EHRLICH'S TRIACID STAIN.—Unsuitable for demonstration of malarial parasites.

JENNER'S STAIN.—Clear pictures of parasites, which, however, show no chromatin; hence unsuitable for study of finer differential points.

NOCHT-ROMANOWSKY METHOD.—Very suitable, but requires accurate mixture of several fluids just before using, which then have to be thrown away.

WRIGHT'S STAIN.—Practically identical with Goldhorn's one-solution stain (*vide infra*), but less rapid; powerful chromatin stain and general blood stain.

POLYCHROME METHYLENE BLUE (GOLDHORN).—To prepare the stain dissolve 1 gram lithium carbonate in 200 c.c. clean water and add 1 gram methylene blue. Shake and dissolve. Pour into porcelain dish over water-bath, stirring frequently until blue color changes to a rich purple. Run through cotton in funnel; make up to 200 c.c. To 100 c.c. add 5 per cent. acetic acid until a faint pink is just visible on litmus paper above level of point discolored by the dye. Now add the remaining 100 c.c. of dye and allow to stand in open dish for forty-eight hours. Run once more through cotton into clean bottle.

I have not found it necessary to use distilled water, and have obtained satisfactory results with all the different forms of methylene blue I have been able to obtain during the past five years. I now prefer B-X Gruebler.

Fix the smear by immersion in commercial wood alcohol for fifteen to thirty seconds; wash well and stain for about ten to fifteen seconds in polychrome; wash and stain for from fifteen to sixty seconds in $\frac{1}{20}$ per cent. aqueous eosin. Wash again in water and dry in air without heat. Body of parasites blue; chromatin is red to purple.

Results may be varied by using polychrome or eosin for different lengths of time. Admirable preparations may be obtained, even when there is precipitation, by just rinsing the smear a little in 50 per cent. ethyl alcohol. This will remove any precipitation.

The simplest method of staining the parasite is probably the following, recommended by me for the staining of mast-cells: Saturate wood alcohol with methylene blue. Pour on dry smear for five to ten seconds and wash in water. Parasite blue.

GOLDHORN'S ONE-SOLUTION STAIN.—To Goldhorn's polychrome methylene blue (*vide supra*) add weak, watery ($\frac{1}{5}$ to $\frac{1}{20}$ per cent.) eosin until the filtrate is of a pale-blue color; the exact amount of eosin will depend upon the degree of alkalinity of the polychrome and upon the amount of unaltered methylene blue in the polychrome.

The precipitate is washed with water and dried without heat and protected from dust. When absolutely dry it is dissolved in commercial wood alcohol, making a 1 to 2 per cent. solution.

The smear is dried without heat and held for a second or two in the dye. It is then dipped slowly into a vessel with clean water, *film side down;* it should not be plunged into the water. The staining depends upon the interaction of the water with the film of dye adhering to the blood. Hold preparation in the water for a few seconds, then move it about for a moment, and rinse in clear water; clean lower side of the slide, as precipitation will have taken place here; hence, do not introduce into water with film side up. Dipping the preparation for a moment into 50 per cent. ethyl alcohol removes smudges and precipitate.

Mode of Infection.—While it is probable that infection with malarial sporozoites occurs in the great majority of cases by the bite of an infected mosquito, there seem to be cases in which the infection has taken place in some other way. Mosquitoes normally feed on plant juices; to obtain their food they insert their proboscis into the plant tissues. Is it then unreasonable to suppose that such a plant would be infected in a manner analogous to the manner in which the human blood is infected? Why should not such a vegetable eaten uncooked cause an infection through the gastrointestinal tract? Again, infected mosquitoes after ovipositing may die in the water, and, partaking of such water, might lead to infection; or if the insect died in the open air the sporozoites might be carried by air currents and infect through the respiratory tract.

The fact that with the extermination of the anopheles varieties malarial fevers in man would be made impossible remains established; the parasite must have its chance of rejuvenescence in the mosquito's stomach.

Hitherto no experiments have been made to show the possibility of sporozoites penetrating the capillary walls of the lung tissue or those of the mucous membranes.

Malarial-like Organisms in Other Animals.—Two varieties of malarial parasites have been found in birds, the proteosoma (*Hæmamœba relicta, Cytasporon danilewskyi*) and the Halteridium (hæmoproteus). The development of each seems to be very similar to that of the human malarial organism. The life cycle of the proteosoma is the better known of the two. The asexual cycle, or schizogony, is best studied in artificially infected birds (canaries). The sexual cycle occurs in the gnat (*Culex pipiens*).

Conjugation of malarial organisms was first observed by MacCallum in the genus Halteridium in birds, and his discovery gave the first clue to the nature of the flagella.

Malarial-like organisms have also been found in monkeys, cattle, and frogs, but they have not been minutely studied.

CHAPTER XLI.

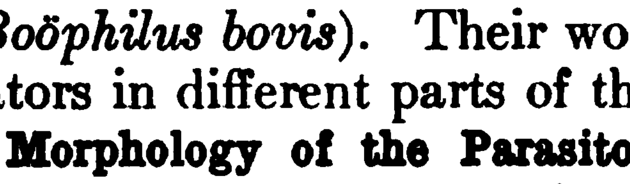
PIROPLASMA BIGEMINUM—THE MICROSPORIDIA—BALANTIDIUM COLI.

Genus Piroplasma Smith and Kilborne (1893).

It was not until 1888 that there was a hint as to the real nature of the actual cause of "Texas fever" and allied diseases which attack field cattle in many parts of the world. Then Babes described inclusions in red blood cells in Roumanian cattle sick with the disease, though he did not decide upon the nature of the organism. No new studies were reported until 1893, when Theobald Smith and Kilborne gave such a complete description of this disease and its cause as occurring in Texas cattle that little that is new has since been discovered.

These authors describe as the cause of Texas fever pigment-free, amœboid parasites appearing in various forms within the red blood cells of infected animals. The organisms may be irregularly round and lie singly or they may be in pear-shaped twos, united by a fine line of protoplasm.

Fig. 157



Piroplasma bigeminum, showing pear-shaped forms in curved and straight axes. (After Kossel and Weber.)

Because of these double pear-shaped forms Smith and Kilborne named the organism pyrosoma bigeminum[1] and placed it among the hæmosporidia. These authors also showed that the contagion was carried by a tick (*Boöphilus bovis*). Their work has been corroborated by many investigators in different parts of the world.

Morphology of the Parasito.—In the examination of fresh blood of sick cattle under 1000 diameters, according to Smith and Kilborne, are seen, in the red blood cells, double pear-shaped forms and single rounded or more or less irregular forms. The size varies, though generally it is the same among the bodies in the same red blood cell. The average size is 2μ to 4μ long and $1\frac{1}{2}\mu$ to 2μ wide. The pointed ends of the double form are in apposition and generally touch, though in unstained specimens a connection between them cannot be seen. The axis forms either a straight line or an angle. The protoplasm has a pale, non-granular appearance, and is sharply separated from the protoplasm of the including red blood cell. The small forms are generally fully homogeneous, whereas the larger ones often contain in the rounded ends a large rounded body, 0.1μ to 0.2μ in size, which is very glistening

[1] The generic name Pyrosoma, already in use for a well-known Ascidian genus, was altered to Piroplasma by Patton in 1895.

and takes a darker stain. Within the largest forms in the centre of the thick end is a large round or oval body, 0.5μ to 1μ, which sometimes shows amœboid motions. The motion of the whole parasite on the warm stage is not produced by the formation of distinct pseudopods, but by a constant change of the boundary. The changes can succeed each other so quickly that it is scarcely possible to follow them with the eye. The motion may persist for hours. The single ones show motion, while the double ones remain unchanged. The parasites take most basic aniline stains well. Methylene blue is especially recommended. The Romanowsky method or its modifications gives the best results.

The number of red cells infected is about 1 per cent. of the whole. If the number increases to 5 per cent. or 10 per cent. it generally means the death of the animal. The parasites quickly disappear from the blood after the disappearance of the fever. In fatal cases many parasites are found in the red blood cells of the internal organs. They vary in number according to the stage at which death occurs, are most abundant in the kidneys, and are found in fewer numbers in the liver, spleen, and other internal organs.

The complete life cycle of the organism is not known. According to Smith and Kilborne tiny motile spore forms enter the red blood cells and divide, the two parts remaining together forming diplococcus-like bodies. These forms increase in size and produce the pear-shaped bodies. How the small forms are produced from these is not known.

R. Koch has described a bacillar form which he found in large numbers in red blood cells of acute fatal cases in East Africa. Between these and the pear-shaped forms he found all grades. He considers them young forms.

Ziemann showed by the Romanowsky method that the parasite contained chromatin staining material situated at or near its periphery.

Kossel and Weber, who studied the parasite found in hæmoglobinuria of cattle in Finland, give the following description of the specimens stained according to Romanowsky:

"The smallest forms appear as tiny rings, about one-sixth the diameter of the red blood cell. The rim of the ring takes the red stain, while the rest appears blue. Forms a little larger are irregular in outline and already show an arrangement of the chromatin into two parts, which are more distinct in larger parasites and which finally become separated into four parts. In the large, double, pear-shaped parasites the chromatin is generally situated at the poles, more seldom near the middle."

No division forms similar to those seen in the sporulating stage or schizogony in malaria have been seen. Neither has a sexual cycle in another host similar to that of the malarial parasite in the mosquito been observed. Smith and Kilborne showed that the infection is caused by the larva of a species of tick, *Boophilus bovis Curtis* (rhipicephalus annulatus), and Kossel gives *Ixodes redivius* as the tick causing transmission of the germ in the hæmoglobinuria of Finland cattle. We know nothing of any changes going on in the parasite in its passage through the tick. The ticks feeding upon the blood of cattle and other mammals become

sexually mature at their last moult. They then pair, and the fertilized females, after gorging themselves with the blood of their host, drop to the ground. Each female then lays about 2000 eggs, and within the shell of each egg a large quantity of blood is deposited to serve as food for the developing embryo. The female then shrivels up, becoming a lifeless skin. The newly hatched larvæ containing in their abdomens some of the mother-blood, crawl about until they either die from starvation or have the opportunity of passing to the skin of a fresh host. If the mother-tick has drawn its supply of blood from cattle infected with piroplasma, her larvæ are born infected with the parasite and become the means of disseminating the disease further. This mode of dissemination explains the long incubation period of the disease (forty-five to sixty days—thirty days for the development of the larvæ and the remainder for the development of the parasite within the host). It is possible that the tick embryo acquires the infection secondarily from the blood it absorbs in the egg, and that the parasites do not pass through the ovum itself as in nosema bombycis.

It is not known whether among the piroplasmata described as occurring in cattle in various parts of the world there are different varieties. They seem to be morphologically very similar and to produce similar diseases. So far it has not been possible experimentally to inoculate animals other than cattle with these parasites. Calves withstand the infection better than older animals and a certain degree of immunity is reached in some of the older cattle in infected districts. The piroplasmata taken in by such animals may remain as harmless parasites for some time. If, however, such cattle are weakened from any cause, their resistance to the organism may be lowered and they may therefore pass through a more or less severe attack of the disease.

Symptoms of the Disease.—Fever (40° to 42° C.), anorexia, weakness, increased pulse and respiration, decreased secretion of milk, hæmoglobinuria at the height of the fever, causing the urine to appear dark red like port wine or darker. The urine may contain albumin even if the hæmoglobinuria is absent, but there are no red blood cells present, the color being due to the coloring matter only. There is icterus of the mucous membrane if much blood is destroyed.

The prognosis varies in different epidemics from 20 to 60 per cent. Death may occur in three to five days after first symptoms appear. Recovery is indicated by a gradual fall of the fever.

The only treatment from which any results have been obtained is quinine in large doses. This seems to have helped in some epidemics.

Prophylaxis.—Stalled cattle are not infected, but it is impracticable to keep large herds of cattle stalled. If the cattle are kept from infected fields for one or two years and other animals (horses and mules) are allowed to feed there the ticks may disappear. The burning of the field for one season may have a good effect. If animals cannot be taken from infected fields such fields should be enclosed.

Ticks on animals may be killed by allowing the cattle to pass through an oil bath (paraffin, cottonseed oil, etc.), whereupon the ticks die from

suffocation. The bath should be repeated after a week in order to kill any larvæ which may have developed. All animals sent from infected regions should receive this treatment. Animals apparently healthy before the treatment, after the disturbing influence of the bath often develop the disease in an acute form and die.

Certain birds in Australia seem to feed on the ticks, therefore such birds might be propagated.

Various attempts have been made to give protection by the inoculation of fresh (not older than two to three days) blood from slightly infected animals. Some partial results have been reported, especially when the inoculations were made during the cold months. In Australia the inoculation of defibrinated blood from animals which have just recovered from the infection, but whose blood still contains some parasite, has been tried. Such inoculations should be followed by a slight attack of the disease in order to give protection. So far no absolute protection has been produced, neither does the parasite-free serum of animals which have entirely recovered from the disease seem to contain protective qualities.

Hæmosporidia similar to those described in the hæmoglobinuria of cattle have been found in dogs, sheep, horses, and pigeons. Nocard and Motas, who have made the most complete study of these parasites in the malignant jaundice or hæmoglobinuria of dogs, state that though the parasites are morphologically similar to those infecting cattle, yet it is impossible to infect cattle or any other animal tried with them. They form therefore a physiological variety.

Piroplasmata in Human Beings.—Recently Wilson and Chowning have reported the infection of man by an organism similar to the piroplasma bigeminum. The cases in which they state that they found the organism were those of "spotted fever" ("black fever," "blue disease") in Montana. According to these investigators the first case of this fever occurred in this vicinity in 1873. Since then probably 200 cases of the severe type have occurred, with a mortality of 70 to 80 per cent. The disease occurs chiefly in the spring. The symptoms are chill; pains in joints; constipation; fever with morning remissions reaching 103° to 104° on the second day of appearance and a maximum of 105° to 107° in five to seven days, diminishing at the end of the second week, and normal two to four weeks later; purpuric eruption over the entire body, appearing from the second to the fifth day after the chill and reaching a maximum in one to two days; slight jaundice; muttering delirium just before death; pulse and respiration very rapid; urine slightly albuminous, with granular and blood casts. The authors studied 23 cases during 1902 and 1903, and in all of these they say that they found the organism, within the red blood cells in most instances, but sometimes between the cells, few in number in some of the cases, many in the others. The time of the appearance of the organism during the course of the disease has not been determined. The authors describe the organism as varying somewhat in size, form, and staining reaction. In general, they state it closely resembles the piroplasma bigeminum.

Inoculation experiments, according to these authors, show rabbits to be slightly susceptible, inasmuch as the organisms cause slight fever and remain for a long time in the blood without apparent harm.

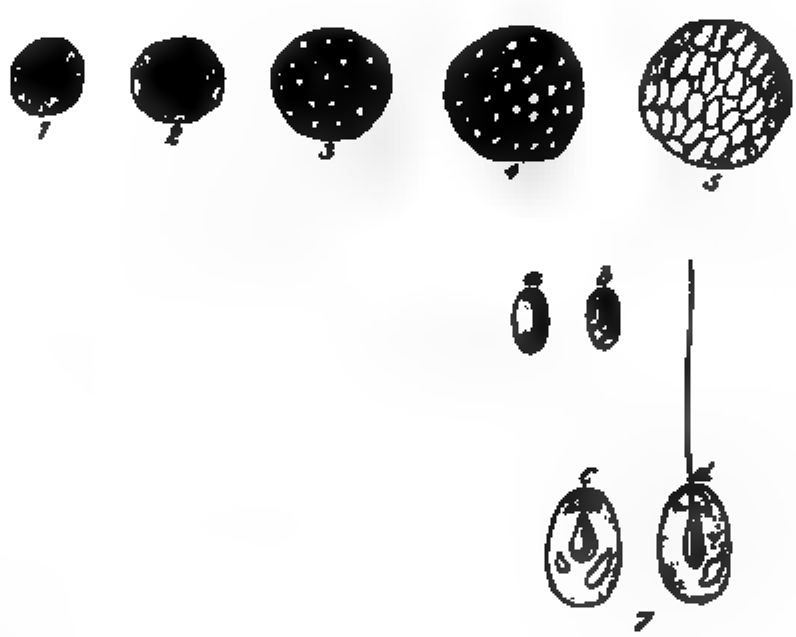
The mode of infection, the authors think, is probably through the bite of ticks, members of the genus *Dermacentor*. These results have not yet been corroborated. On the contrary, some good authorities state that the bodies described by Wilson and Chowning as organisms are artifacts, and that there is no evidence to show that the disease in question is caused by a protozoan.

Cnidosporidia.

Subclass: Neosporidia.
Order: Cnidosporidia.
Suborder: Microsporidia.

The cnidosporidia, or, as the whole group is still sometimes called, the myxosporidia, is one of the most populous and abundant groups of the sporozoa, showing great structural variation as well as divergence in mode of life. Nevertheless the members have as a group the follow-

FIG. 156



Nosema bombycis: 1 to 5, spore formation; 6, infected follicle of testicle; 7, spores; *a*, *b*, fresh; *c*, *d*, treated with nitric acid. The acid causes them to swell up and increase in size by at least a half, at the same time making the polar capsule distinct. In *d* the filament is extruded. (After Balbiani.)

ing well-marked characteristics: The trophozoite is amœboid; spore formation begins at an early period and proceeds continuously during the growth of the trophozoite; the spores are produced endogenously—*i. e.*, within the protoplasm of the trophozoite, and each spore always possesses one or more very distinctive structures, "the polar capsules." The cnidosporidia are habitants of fishes, reptiles, arthropods, and some other classes of animals.

The microsporidia infest especially arthropods, causing often most virulent epidemics. The most interesting member of this group is

Nosema bombycis, the cause of silkworm disease (Pébrine). The organism forms many small spores each with one polar capsule. The spores are carried by the food into the intestinal canal of the caterpillar, pass through the walls of the intestines, and infect all organs. Spores found in the ovary may be carried over to the newly hatched silkworms, thus causing a further dissemination of the disease.

The other member of this group of interest here is *Nosema lophii* (Doflein). Its interest lies in the fact that it has been found to infect only the ganglion cells of the sea-devil, thus apparently resembling in its parasitic nature the organism causing hydrophobia.

Heterotricha.

Class: Ciliata.
Order: Heterotricha.
Genus: Balantidium.

Balantidium coli (Malmst, 1857). The body of this infusorium is egg-shaped, with a funnel-shaped mouth opening. The surface of the body is covered with a pellicula, under which is a distinct ectoplasmatic sheath containing rows of basal granules from which the short, fine cilia arise.

FIG. 159

1 2 3

Balantidium coli 1, 2, stages of division, 3, conjugation. (After Leuckart.)

The cloudy entoplasm contains fat and starch granules and may contain many red blood cells and other food particles from the host. Two contractile vacuoles have been seen. Posteriorly there is a small prominence marking the place where excreta are expelled. The chromatic macronucleus is bean-shaped, and the vesicular micronucleus is nearly spherical.

Division is transverse, the macronucleus dividing by simple constriction and the micronucleus by mitosis. Conjugation has been observed. Spherical cysts surrounded by a thick membrane are formed.

The balantidium coli has been found in the large intestine of human beings and of swine—probably two distinct varieties. The variety

occurring in human beings has been found in about 60 cases, principally in Sweden, but also in Russia, Scandinavia, Finland, China, Italy, Germany, and the United States. Most of these cases were suffering from severe chronic intestinal catarrh, often accompanied by bloody diarrhœa. A number of observers think the balantidium the primary cause of the catarrh, others think it a secondary excitant, while still others believe it to be a harmless inhabitant of the intestines.

Schaudinn has described two additional species of balantidium found in the human intestines, which he has called *Balantidium minutum* and *Nyctootherus faba*, probably both non-pathogenic.

CHAPTER XLII.

PROTOZOAN-LIKE BODIES IN SMALLPOX AND ALLIED DISEASES (COWPOX, HORSEPOX, SHEEPPOX) AND IN SCARLET FEVER.

THE question as to the chief exciting factor in smallpox and allied diseases, according to some authorities, is still undecided, while according to others it is settled beyond doubt. These latter investigators consider that certain bodies found chiefly in the epithelial cells of the skin and mucous membranes in the specific lesions of these diseases are protozoa causing the diseases.

The different diseases named in the chapter heading, excepting only scarlet fever, if not identical, are closely allied. Indeed, the following facts seem to prove that at least cowpox and variola are very closely related diseases, if not the same disease: First, smallpox virus inoculated into calves produces after passage through several animals an affection exactly similar to cowpox. The successful inoculation of the first series of cattle from smallpox is a matter of great difficulty, but so many experimenters have asserted that this has been done that there seems to be no doubt as to its truth. In our laboratory not one of many attempts to accomplish it has been successful. Second, both when occurring in nature and when produced by experiment the lesions of the two diseases are similar. Third, monkeys have been successfully protected against either disease by previous inoculation of the other; also, observations go to show that human beings inoculated with cowpox vaccine are not susceptible to inoculation with smallpox virus, and that those who have within a varied time passed through an attack of smallpox cannot be inoculated successfully with cowpox vaccine. These facts seem positively to prove that the two diseases are produced by organisms originally identical, one being modified by its transmission through cattle, the other through human beings.

The immunity caused by successful vaccination is not permanent, and varies in its duration in different individuals. Although it may give some protection from smallpox for ten or fifteen years, it is not well to count on immunity for more than two years, and whenever one is liable to exposure it is well to be vaccinated. If this vaccination were unnecessary it will not be successful, while if it is successful we have reason to believe the individual was open to at least a mild smallpox infection.

Protective Substances Present in the Serum of Animals after Successful Vaccination.—It has been frequently shown that the blood serum of a calf for several weeks after successful vaccination possesses feeble protective properties, so that the injection of one to two litres of it into a susceptible calf would prevent a successful vaccination. A further

and more convincing fact has been demonstrated by Huddleston and others, namely, that when active vaccine is mixed in certain proportions with serum from an animal which had just recovered from a successful vaccination, and the mixture is inoculated into a susceptible animal, there is no reaction.

Etiology of Variola and Cowpox.—It has been repeatedly shown that no bacteria similar to any of the known forms have a causal relation to these diseases. In our own laboratory we are able, by the inoculation of rabbits' skins, to produce extremely active vaccine virus in large quantities, absolutely free from micro-organisms as grow under the conditions of our present methods of bacterial cultivation. Such pure active vaccine, when emulsified in equal parts of glycerin and water and filtered through two or three thicknesses of the finest filter paper, gives a slightly opalescent filtrate, which in the hanging drop under high magnification shows many very tiny granules with an occasional larger one, and in smears shows no formed elements giving characteristic stains. This filtrate, from which no growth can be obtained on artificial culture media, when rubbed over a freshly shaved rabbit's skin, after the method of Calmette and Guérin, gives an abundant typical reaction.

These facts show that some, at least, of the infective forms cannot as yet be made to grow outside of the body, that such forms are very tiny, and that they do not stain characteristically with our usual methods of staining. We have shown also that the infective agent cannot pass an ordinary Berkefeld filter under forty pounds' pressure, which practically rules out ultramicroscopic forms.

Since Guarnière in 1892 claimed that certain inclusions present in the epithelial cells of the lesions of smallpox in a rabbit's cornea (Fig. 160) were parasites, much attention has been given to the study of these bodies, commonly known as "vaccine bodies," yet opinions still differ as to their nature. The most recent studies of importance of these bodies have been made, on the one hand, by Councilman and his associates, who believe them to be protozoa, and, on the other, by Ewing, who believes that all of the forms so far described are degeneration products, some specific, others not.

Councilman believes that there are two cycles of development of the "parasite," one intracellular and the other intranuclear, and that the intranuclear infection occurs only in smallpox. The intracellular cycle is simple, showing only "multiplicative reproduction," while the intranuclear cycle is more complicated, probably sexual in character. Calkins, working with Councilman, has described an elaborate cycle of development in which we believe are included many forms due to degeneration of the host cells alone.

In our own work on sections, which has extended irregularly over a period of several years, we have gotten results which are somewhat confusing, principally so because of the non-uniformity of the appearances of these bodies, both by different methods and by the same methods at different times. There is no doubt that whatever the nature of the bodies they are easily affected by methods used for fixing, hardening, and staining

them. This accounts in part for the varied results reported. However, in the most perfectly prepared specimens, judged according to the appearance of the red blood cells, leukocytes, and tissue cells at a distance from the lesions, we have found the vaccine bodies, especially in corneal

FIG. 160

Epithelial cells of a rabbit's cornea, containing "vaccine bodies." Tissue fixed three days after inoculation with smallpox virus. *a* and *d*, vaccine bodies; *b* and *c*, nuclei. × 1500 diameters.

infection, to show a more or less constant series of changes, somewhat similar to those described by Calkins in his "gemmule formation" and by Tyzzer in his development of the vaccine bodies. This series of changes might be represented somewhat schematically in Fig. 161.

FIG. 161.

Schematic representation of vaccine bodies seen within the epithelial cells in the lesions of smallpox and vaccinia: 1, spore (merozoite, sporozoite?), 2, small form which stains solidly with basic stains; 3, larger form which contains central, more darkly staining granule; 4, larger form, with more lightly staining reticular cytoplasm. This form and the next may have amœboid outline, and there may be larger amœboid forms which might be interpreted either as the grown single form or as the fusion of two or more forms; 5, form containing two central, darkly staining bodies; 6, form containing many bodies taking basic stains more or less intensely; 7, form containing a central body staining faintly with basic dyes, and small rounded bodies about it, some taking basic and some acid stains; 8, same as 7, except that many of the bodies surrounding the central body are definitely ring-shaped, and all take the acid stain. These forms vary in size; some are larger than the host nucleus; 9, form breaking up (spores set free?).

One can easily see that such tiny bodies as these possible spores with no definite characteristic staining qualities would be with difficulty, if at all, differentiated from the mass of cell granules in the degenerated areas of the lesion, and, as the outline and structure of most of the other forms seem to be easily disturbed, the whole question as to their nature is, from a morphological standpoint alone, a very diffcult one to settle.

Our best results on corneas have been obtained with the following technique: Fix in Zenker's fluid for from four to eight hours; wash in running water over night; place in 95 per cent. alcohol (changing in two hours to fresh) for twenty-four hours, then in absolute alcohol for twenty-four hours. Imbed in paraffin. The cuts should be from 3μ to 5μ thick. Stain with (1) eosin and methylene blue—eosin half an hour, methylene blue two minutes; (2) Heidenhain's iron hæmatoxylin; (3) Bovrel, modified by Calkins.

Susceptibility of Different Animals.—Horses, rabbits, sheep, monkeys, and guinea-pigs are susceptible. The pulp and serum obtained from an epidemic of cowpox took feebly in calves in a moderate percentage of those inoculated. The characteristic vaccine bodies were found practically identical with those in vaccinia, except that the bodies were a little larger and more irregular in outline.

The Preparation of Vaccine.—For the following suggestions I am indebted to Dr. J. H. Huddleston, who has had the immediate charge of the production of vaccine for the New York Health Department for some years:

SEED VIRUS.—A sufficient amount of vaccine virus should be on hand to vaccinate forty to fifty persons. Five children in good health, and not previously vaccinated, should then be vaccinated each in a spot the size of a ten-cent piece. On the fifth day after vaccination the top of the resulting vesicle should be removed and sterilized bone slips be rubbed on the base thus exposed. It should be possible in this manner to charge at least from one to two hundred slips on each side of the slip from each child. The slips should be allowed a moment in which to dry and then placed in a sterilized box, in which, if kept in cold storage, they will probably remain efficient for at least two or three weeks.

ANIMALS.—The preferable animals are female calves, from two to four months of age, in good condition and free from any skin disease. These can easily be vaccinated on the posterior abdomen and inside of the thighs by placing them on an appropriate table. It is possible that on account of the character of the available supply older animals may be desirable, but the calves take more typically and are more easily handled. When an animal is too old to be thrown and held without difficulty it may be vaccinated on the rump, each side of the spine; but the skin there is tougher than on the posterior abdomen and inside of the thighs, and the resulting virus, though efficient, is not so easily emulsified.

VACCINATION.—The calf should be cleaned thoroughly, including the feet and the tail, and the hair should be clipped from the end of the tail. Next, the posterior abdomen and insides of the thighs are shaved and the skin beneath washed in succession with soap and water, sterilized water and alcohol, and then dried with a sterile towel. On this area there are now made about one hundred scarifications, each from one-quarter to one-half of an inch square. The scarification is made most easily by cross-hatching with a six-bladed instrument, the blades being about one-thirtieth of an inch apart. The scarification is super-

ficial, but brings a small amount of blood. An area as small as specified is less likely to become infected than a larger one. The scarifications should be separated from each other by an interval of at least one-half to three-quarters of an inch. After they have been made they should be dried with a sterile towel or with sterile cotton and rubbed with the charged slips. One to two slips, depending on the amount of virus each slip holds, should be sufficient for vaccinating each vesicle.

Collection.—On the fifth or sixth day, depending upon the rate of development of the vaccine vesicles, they should be ready for collection. The entire shaved area is washed with sterile water and sterile cotton, and the crusts are picked off. The soft, pulpy mass remaining is then curetted off with an ordinary steel curette and the pulp placed in a sterilized vessel. After the curettage, serum exudes from the torn base of the vesicle, and ivory slips may be charged in this. The pulp should be mixed with from two to three times its weight of glycerin and water, equal parts, and this is done most effectively by passing the mixture between the rollers of a Doring mill. The more watery the pulp, especially if it is not to be used immediately, the smaller should be the proportion of glycerin. The emulsion so produced can then be put up for issue in vials. The slips charged with the serum from the calf may also be used for vaccinating. Capillary tubes require especial means of filling, and small vials filled and corked answer the purpose admirably.

Propagation.—Subsequent animals may be vaccinated in any one of the three ways: (*a*) slips may be charged from typical vesicles on primary vaccinations, just as with the first calf, and used for seed virus; (*b*) slips charged with the serum from the calf may be used to vaccinate a second calf; (*c*) the glycerinated emulsion may be used to vaccinate succeeding calves, but in the last case it is necessary to keep the emulsion a varying length of time—often two or three months—before it is fit for use in vaccination of the calf, since the employment of fresh glycerinated pulp on a succession of calves leads to prompt degeneration of the vaccine and to the production of infected vesicles.

Care of the Calves.—All bedding is avoided and an exclusively milk diet given; thus much of the otherwise unavoidable dust is done away with.

Laboratory.—The laboratory should consist of at least three rooms: (*a*) stable; (*b*) operating-room; (*c*) laboratory-room. It should be possible to make and keep all the rooms clean. The stable and operating-room should be flushed with a hose and hot water daily. Excreta should be removed immediately. The calves can be kept clean if they stand on a raised and perforated platform, which is so short that the defecations cannot fall on it, and if they have no bedding. They must be fastened to keep them from licking the scarifications. In the health department, when a calf is removed, its stall and platform are scoured with a brush and sodium carbonate solution. The stable should be provided with a shovel, broom, hose, currycomb, mane brush, cord, and with halters, buckets, scrubbing brushes, and sponges. The

operating-room should be well lighted and provided with a table and with stools.

The only requisites for the table are that it should be heavy and firm; that it should have holes through the top so arranged that straps can be passed through them to hold the calf down, and a vertical strip on one side of the table to which the upper hind leg of the calf can be fastened. The calf can be thrown upon the table easily by two attendants.

The laboratory should also be well lighted and furnished with tables, chairs, desk, case for instruments, and refrigerator. It should also have both a steam and a dry-air sterilizer, a set of scales weighing to grams or centigrams, and a blast lamp and bellows. In stock there should be one to two thousand bone slips for seed virus, and ten to fifteen thousand smaller slips for issue; two or more scarifiers; a curette; four to six razors for shaving the animals; a razor strop; a pair of large scissors, curved on the flat, for clipping the animals; a burette, from which glycerin flows while the vaccine pulp is being ground; burette holder; a Doring vaccine grinder; clinical thermometers to take the temperature of the animals; six to twelve small glass dishes with covers; a hard-rubber syringe, of four-ounce capacity, to make suction; absorbent cotton; glass vials and corks; and several pounds of soft glass tubing, three-eighths of an inch in calibre, to store virus emulsion. There should also be gowns and caps for the attendants. Sodium carbonate, bichloride of mercury, bromine for a deodorizer, alcohol, and glycerin are the chemicals needed.

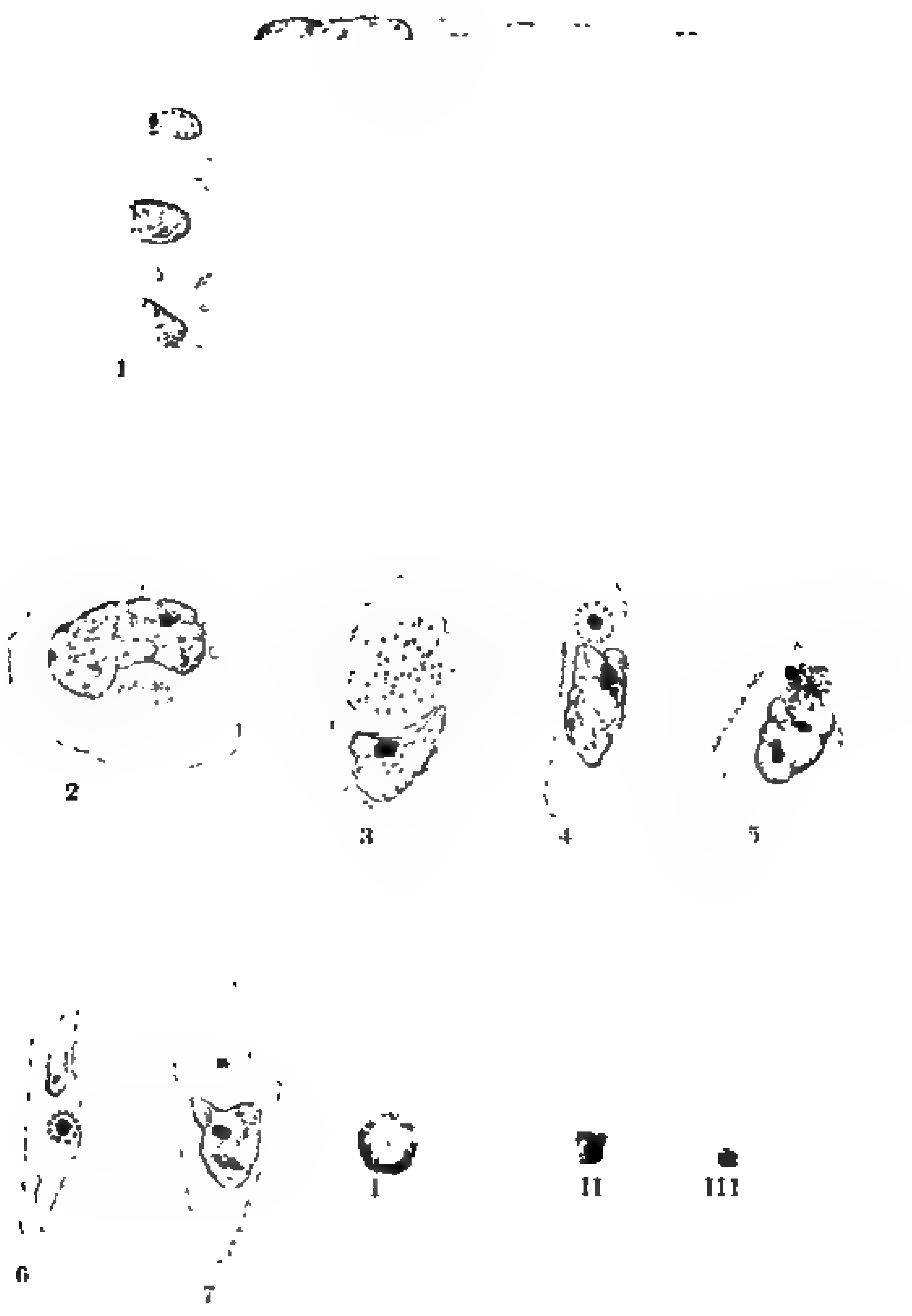
For issue for public vaccinations there are also needed packing-boxes, rubber bands, sheet wadding, needles, and wooden toothpicks for removing the virus from the vials and rubbing it on the scarifications.

Yield.—The material obtained from the five children should vaccinate at least five calves; it may easily vaccinate fifteen calves. Ten grams of pulp and two hundred charged slips would be an average yield from a calf, and that, when made up, should suffice to vaccinate at least fifteen hundred persons. Calves vary immensely in the yield. Of two calves vaccinated in precisely the same way one may furnish material for five hundred vaccinations and the other for ten thousand vaccinations.

The Durability of Glycerinated Virus in Sealed Tubes.—As a result of testing from time to time an immense number of specimens of vaccine, the conclusion has been reached that vaccine properly put up should keep at least three months. From time to time a single lot of virus will fail by the end of one month. Sometimes this is due to the glycerin, as when it has some chemical impurity, or as when simply it is not diluted sufficiently with water. We find that one part of water to two of glycerin makes a good dilution.

Bacteria in Vaccine.—It is impossible to prepare vaccine on a large scale so that it is at the time of its removal free from bacteria. In fact, there are usually very large numbers of one or more varieties of bacteria

PLATE IV.



Protozoan-like Bodies in Scarlet Fever (1-7) and Measles (I-III).

present. When the stable and animals have been kept clean the bacteria comprise usually very few varieties; when dirty conditions prevail the bacterial varieties are more numerous. The number of bacteria found varies enormously. The largest number found by us in vaccine pulp from the calf was 126,360 in one loopful, and the smallest number 523. Discrete vesicles at the borders contain many less bacteria than the confluent ones caused by the inoculation at the scarification. The pulp has many more bacteria than the serum of the vesicles. The period which elapses before glycerinated virus becomes sterile is also quite variable, but does not depend in any direct way upon the number of bacteria originally present. A very large number may disappear rapidly, and a few persist for a long time. Upon rabbits a practically bacteria-free vaccine can be obtained.

After two or three weeks the number of living bacteria is usually greatly diminished, especially after addition of glycerin, but seldom is absolutely *nil*. If we wait until the vaccine is surely sterile it is very apt to be also useless—that is, by that time the specific organisms, too, as well as the bacteria, are dead.

In a very large experience we have learned that the number of bacteria present has little to do with the result of the vaccination.

Pathogenic bacteria other than the practically non-virulent skin staphylococci are not found when animals are properly kept and vaccinated. The vaccine pulp and serum mixture is added to two and one-half to three and one-half times its bulk of a mixture consisting of two parts of chemically pure glycerin and one part of water.

Efficient vaccine should be inoculated in a portion of skin no more than one-eighth inch in diameter.

Scarlet Fever.

Very recently Mallory has reported the presence of certain bodies in scarlet fever. He summarized his observations as follows: "In 4 cases of scarlet fever certain bodies were found which in their morphology strongly suggest that they may be various stages in the developmental cycle of a protozoan. They occur in and between the epithelial cells of the epidermis and free in the superficial lymph vessels and spaces of the corium. The great majority of the bodies vary from 2μ to 7μ in diameter, and stain delicately but sharply with methylene blue. They form a series of bodies, including the formation of definite

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Description of Plate IV.

Photographs of three forms of the small bodies found in blister fluid from cases of measles.

I. Small form with central chromatin mass.

II. Medium-sized form with chromatin granules distributed throughout its protoplasm.

III. Large form with most of the chromatin granules peripherally arranged.

Protozoan-like bodies in scarlet fever. Stained with eosin and methylene blue. 1, numerous large and small scarlet fever bodies (stained light blue) in and between the epithelial cells of the rete mucosum. Several of the bodies suggest fixation while in amœboid motion; 2, coarsely reticulated form which may be degenerated form or stage in sporogony; 3, probable stage preceding the radiate bodies; 4, 5, 6, and 7, different stages in the development of the radiate bodies. (After Mallory.)

rosettes with numerous segments, which are closely analogous to the series seen in the asexual development (schizogony) of the malarial parasites, but in addition there are certain coarsely reticulated forms which may represent stages in sporogony or be due to degeneration of the other forms." In our laboratory nine cases have been examined with reference to the presence of these bodies, five autopsy cases and four living, but so far only a few of the less characteristic forms have been found, and these only in the five autopsy cases.

Duval has just made the announcement that in fluid obtained through blistering the skin of scarlet fever patients by a very quick method he has obtained bodies which he interprets as forms of Mallory's protozoan.

In our laboratory Field has obtained similar bodies by the same method in both scarlet fever and measles cases, and in four cases of scarlatiniform antitoxin rashes, more in the first two groups than in the last. He has obtained them in no other cases so far examined. Nothing positive can be said yet as to the nature of these bodies, though Field has come to the conclusion that the majority of them are from degenerated leukocytes.

CHAPTER XLIII.

KALA-AZAR—RABIES.

Kala-azar.

CERTAIN fevers of severe malarial-like types known in different sections of the tropics by different names (dum-dum fever, cachexial malaria, kala-azar) have recently been shown to be due to the same cause by the finding of similar protozoan-like bodies in the lesions. These bodies were first minutely described by Leishman in 1900 as being present in certain cells in the spleen of cases called by him dum-dum fever occurring in India. In 1903 Donovan described similar bodies in cases of what he called malarial cachexia. The bodies have been called the Leishman-Donovan bodies or, more properly, the Leishman bodies. They have since been found in different parts of India, in China, Tunis, Algiers, Arabia, and Egypt, and quite recently Wright has reported in a case of Delhi boil from Armenia bodies which, according to his excellent photographs and description, must be identical with or very closely related to Leishman's bodies, though he does not call attention to that fact.

The bodies have been found in large endothelioid cells in the spleen, liver, mesenteric glands, bone-marrow, kidney, lungs, testes, skin, and ulcers in intestines.

The symptoms, in the cases of general infection are: (1) very much enlarged spleen and less enlarged liver; (2) progressive anæmia with peculiar earthy pallor of skin, progressive emaciation, and muscular atrophy; (3) long-continued, irregularly intermittent fever (97° to 104°); (4) hemorrhages, such as epistaxis, bleeding from gums into subcutaneous tissue, producing purpuric eruption; (5) transitory œdemas of various regions. There are often complications such as congestion of lungs, dysentery, cancrum oris. The blood count shows practically no loss of hæmoglobin, but there is a decrease in the leukocytes, principally polynuclears, giving a relative increase of mononuclears.

Negative points which help in the diagnosis are: absence of malaria, no typhoid or Malta fever reaction, resistance to medication, quinine, as a rule, having no effect, though in early cases a few good results have been reported. Splenic puncture with the finding of Leishman bodies makes the diagnosis certain. The duration of the disease is from a few months to several years. The percentage of deaths is great; in some forms of the disease it may reach 90 per cent.

Morphology.—The bodies are circular to elliptical in shape, from 2μ to 4μ in diameter, and contain a double nucleus, a large oval one at one part of the periphery and a small circular or rod-shaped one near or at the

opposite part of the periphery. This smaller body stains more intensely than the larger one, while the cytoplasm of the parasite stains very dimly, sometimes showing only a faint peripheral rim. Any nuclear and cytoplasmic staining methods will bring out these points in Zenker fixed material. Smears stain well by Wright or the Nocht-Romanowsky method. The large cells containing the parasites are supposed by Christopher to be the endothelial cells from the finest capillaries. Leishman, Marchand, Rogers and others think the bodies one stage in the life cycle of a flagellate, possibly a trypanosome, Rogers and Marchand having seen flagellates develop from infected tissue *in vitro* in non-coagulable blood. Donovan, however, claims to have found small forms in the red blood cells in the peripheral circulation when

FIG. 162

Protozoa in a case of tropical ulcer. Smear preparation from the lesion stained with Wright's Romanowsky blood-staining fluid. The ring-like bodies with white central portions and containing a larger and a smaller dark mass are the micro-organisms. The dark masses in the bodies are stained a lilac color, while the peripheral portions of the bodies in typical instances are stained a pale robin's-egg blue. The very dark masses are nuclei of cells of the lesion. × 1500 approximately. (After Wright.)

the temperature was above 103°, and his observation has been confirmed by Laveran and Mesnil, who believe the organism to be a piroplasma. Segregation is recommended as the best means of eradicating the disease.

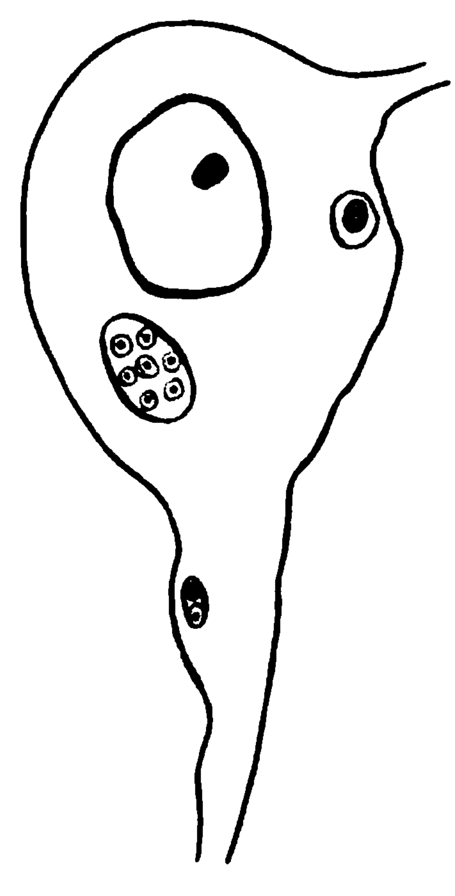
Rabies (Hydrophobia).

Rabies is an acute disease of animals, dependent upon a specific virus, and communicated by inoculation to man. It is usually associated with an injury, such as the bite of a dog, or the inoculation of the broken surface with the saliva of an animal affected with the disease.

This is the so-called rabies of the streets. Wolves, cats, foxes, dogs, horses, cows, and deer may contract the disease; monkeys, rabbits, and guinea-pigs are all inoculable with it, as, indeed, are all warm-blooded animals. Rabies occurs in almost all parts of the world; it is most common in Russia, France, and Belgium; it is not infrequent in Austria and in those parts of Germany bordering on Russia, and in England. In this hemisphere it is comparatively rare, yet it occurs occasionally in various parts of the United States, in Mexico and South America. It is extremely rare in North Germany, Switzerland, Holland, and Denmark, owing to the wise provision that all dogs shall be muzzled; and in Australia it has not been known to occur.

Etiology and Pathogenesis.—Until recently all of the numerous researches in regard to the specific cause of rabies gave negative results. The latest studies of this disease, however, make it seem probable that hydrophobia may be added to the growing list of diseases caused by protozoa. In 1903 Negri described certain bodies in the large nerve cells throughout the central nervous system of animals dying of this disease. He found them in largest numbers in animals dying after the fourteenth day of the disease, and especially numerous in the cells of the gray matter of Ammon's horns. He did not attempt to study the bodies very minutely. Besides demonstrating them in dogs, he found them in rabbits, in a cat, and in a human being. He considers the bodies protozoa and the cause of rabies. In our laboratory we have fully corroborated Negri's results. There is no doubt as to the presence of the bodies in large numbers in animals in which the disease has progressed somewhat slowly. In animals dying quickly of fixed virus we have also found many, but the forms are very small, so that they may easily be overlooked in poorly prepared specimens. We have seen bodies small enough to pass a Berkefeld, thus accounting for the positive results obtained by some observers after the inoculation of filtrates obtained from Berkefeld filters. We have found the larger forms especially numerous in the brains of guinea-pigs inoculated with street virus. Since the discovery of these bodies Poor, in the Health Department laboratory, has examined 23 cases of "street rabies," 17 in dogs, 3 in horses, and 2 in human beings. In all of these cases he demonstrated the presence of the Negri bodies in sections of the central nervous system. All of these cases were subsequently proven to be hydrophobic by animal inoculation. In a number of suspicious cases, which were afterward proven to be non-rabic, no bodies were found.

FIG. 168



Negri bodies within nerve cell of cornu ammonis in rabies. (Schematic.)

The smallest bodies in the sections seem to be structureless, taking a homogeneous purplish stain with eosin and methylene blue. As the bodies become larger they present a definite structure. A central granule

surrounded by a slight space appears, which may or may not take a deeper stain. In the larger forms there are more than one of these granules. As many as eight have been seen. These may be arranged more or less regularly about a central larger one, or scattered less regularly throughout the substance of the body.

We have also identified these bodies in smears of infected brains by fixing the smear in methyl alcohol, and staining for twenty-four hours by the Nocht-Romanowsky method as recommended by Ewing. The bodies stain a robin's-egg blue or lilac, with dark-blue, more or less regularly arranged granules or rings, one in the smaller bodies and more in the larger, corresponding with the structure of the bodies seen in sections. So far no similar bodies have been seen in control smears, and it is evident that this might prove a reliable method for quick diagnosis; at least, in cases where these bodies are fairly numerous. It seems to us possible that the bodies are protozoa and that they are the cause of rabies. Their diagnostic value is certain.

The bulk of the toxic material outside of the central nervous system appears to be excreted in the saliva of the submaxillary gland, though a certain small quantity may be excreted by the other salivary glands, and also by the lacrymal glands, the pancreas, and the mammæ of rabid animals. The poison may also be found in the suprarenal bodies and the peripheral nerves. It has not been found in the blood, the urine, or the aqueous humor of the eye; it has been reported to have been found in the fœtus.

That the disease is due to some form of organism which has the power of multiplying in the tissues and of producing a toxic substance, which appears to act specifically upon the central nervous system, cannot be doubted. As in other specific infectious diseases, the virus is transmitted directly from animal to animal through the medium of some fluid or secretion; it is now very generally recognized that the disease cannot arise anew, as was at one time assumed. In rabies, again, as in other infectious diseases, there is a period of incubation during which the poison appears to increase in quantity.

The certainty with which the disease may be produced and its severity have been found to be governed by three factors: (1) the quantity of the rabic virus introduced; (2) the point of inoculation; (3) the strength of the virus as determined by the kind of animal which affords the cultivation ground for the growth of the hypothetical organism. It is a matter of common observation in hydrophobia of man that slight wounds of the skin, of the limbs, and of the back are often followed by the disease after an extremely long period of incubation; while in lacerated wounds of the tip of the fingers, where small nerves are numerous or where the muscles and nerve trunks are reached, or in lacerated wounds of the face, where there is a similar abundance of nerves, the period of incubation is usually much shorter and the disease generally much more rapid. Experimental infection in animals is produced with the greatest certainty when the material from the nerve centre (the spinal cord or bulb) of an infected animal is injected into

the dura mater of the brain. It may be produced almost as certainly when the infection is made into the anterior chamber of the eye or into the greater nerve trunks. Intravenous injection is usually followed by positive results in small animals, but the larger animals do not succumb to this mode of inoculation. Subcutaneous inoculation in animals is uncertain, because the peripheral nerves are not always injured; but injection directly into a mass of muscle, especially into parts which are rich in nerves, almost invariably produces the disease. Absorption of the rabic poison, even from a healthy mucous surface, has been said to have taken place; and the conjunctiva, the nasal and genital mucous membranes, and the digestive tract have been noted as unabraded surfaces from which this has occurred. The rapidity with which the virus is diffused through the body from the point of inoculation in the tissues seems to vary according to the location of the wound, but it is always comparatively slow. It has been found that rabbits, when etherized and then presented to a mad dog to be bitten on the fur, escape the disease in a very large proportion of cases, although the teeth may have passed well through the skin; if, on the other hand, the part presented to the rabid dog be shaved before it is bitten, the bitten animals contract rabies in a much larger proportion of cases. So in man, in many cases the rabic virus may be cleaned from the teeth by the clothing which covers the bitten part before they come in contact with the skin. From what has been said it is evident also that when the skin is thick and the nerves few a small quantity of virus may find its way into a wound, but not penetrate into the nerves, and thus the person bitten by a rabid animal may escape without any ill effects beyond those due to a lacerated wound. This will explain the fact that only about 16 per cent. of the cases bitten by rabid animals appear to contract hydrophobia.

Preventive Inoculation against Rabies.—The old treatment of rabies consisted simply in encouraging bleeding from the wound, or in first excising the wound and then encouraging bleeding by means of ligatures, warm bathing, cupping-glasses, etc.; the raw surface was then freely cauterized with caustic potash, nitric acid, or the actual cautery. It is doubtful whether the disease ever manifests itself after such heroic treatment if the wound be small; but when the wounds were numerous or extensive the mortality from it was still high. As it was often impossible to apply cauterization to the wound rapidly or deeply enough to ensure complete destruction of the virus, Pasteur and others were led to study the disease experimentally in animals, with the hope of finding some means of immunization or even cure through bacteriological technique; these investigations finally resulted in the discovery of methods of preventive inoculation applicable to man.

Immunization against rabies may be effected in several different ways. Pasteur's treatment is based upon the fact that rabic virus may be attenuated or intensified for any animal at will. He first observed that the tissues and fluids taken from rabid animals varied considerably in their virulence. Then he showed that the virus taken from similar

positions—say, the cerebrospinial fluid—had always the same action in the same species; but that fluid taken from an animal of different species was weaker or stronger as the case might be. Thus the cerebrospinal fluid of a series of dogs is of constant strength and inoculations made from dog to dog regularly produce death from rabies, the animals passing through an incubation period fairly constant in length, and through a series of similar symptoms up to death at the expiration of the same term. If, however, a series of monkeys be inoculated the virus gradually becomes attenuated, and this attenuation becomes more and more marked in successive inoculations until eventually, after the disease has run a longer and longer course in the successive animals, there comes a time at which the virus is no longer sufficiently active to cause death. If this attenuated fluid be now passed through a series of rabbits, dogs, or guinea-pigs it comes back to such a strength that it will kill, though slowly; then, however, its virulence gradually increases until the original intensity is reached. If successive inoculations be made into rabbits with fluid, either from the dog or the monkey, the virulence may be so exalted beyond that of the virus taken from a street dog, in which the incubation period is from twelve to fourteen days, that at the end of the one hundredth passage the incubation period may be reduced to about six or seven days. This, the strongest virus obtainable, was called by Pasteur the "fixed virus." Rabic virus appears also to become attenuated under certain conditions of temperature; indeed, if it be subjected for about an hour to 50° C., or in half an hour if to 60° C., its activity is completely destroyed. A 5 per cent. solution of carbolic acid, acting for the same period, exerts a similar effect, as do likewise 1:1000 solutions of bichloride of mercury, acetic acid, or potassium permanganate. The virus also rapidly loses its strength by exposure to air, especially in sunlight; when, however, protected from heat, light, and air it retains its virulence for a long period. In his earlier experiments Pasteur selected a series of rabic poisons of different strengths, beginning with that obtained from the spinal cord of the monkey—from the very weak to the strongest that he could obtain in this animal—then passing through a similar series obtained during the process of exaltation of the virus by passage through the rabbit. By inoculating dogs subcutaneously with virus taken from a series commencing with the weakest taken from a monkey, and gradually working up to that obtained from the rabbit—from the earliest to the latest in the series—the animals become immune not only against subcutaneous injection, but against subdural infection with fixed virus, and also against the bite of rabid dogs. Such a method as this, however, had several disadvantages, and was not absolutely certain in its action, as only fifteen out of twenty dogs were completely protected. Pasteur, therefore, assisted by Chamberland and Roux, devised a more trustworthy and accurate method, in which he utilized the fact that the cord of a rabic animal when kept under certain conditions loses its virulence in fourteen days. A series of cords cut into short segments, which were held in series by the dura mater, were suspended in sterile glass

flasks plugged with cotton stoppers, and containing a quantity of some hygroscopic material, such as caustic potash; and the whole was kept at a temperature of about 22° C. The cord when taken out at the end of the first twenty-four hours was found to be almost as active as the fresh untreated cord; that removed at the end of forty-eight hours was slightly less active than that removed twenty-four hours previously; and the diminution in virulence, though gradual, progressed regularly and surely until, as already noted, at the end of the fourteenth or fifteenth day the virus was inactive. An emulsion of the cord of the last day was made, and a certain quantity injected into a dog that had been bitten; this was followed by an injection of an emulsion of a thirteenth-day cord, and so on until the animal had been injected with a perfectly fresh and, therefore, extremely active cord, corresponding to the fixed virus. Animals treated in this way were now found to be absolutely protected, even against subdural inoculation with considerable quantities of the most virulent virus; and thus Pasteur's protective inoculation against rabies became an accomplished fact. As it would be impossible, however, or very undesirable, to inject any but persons who had actually been bitten by a rabid, or presumably rabid, animal, Pasteur continued his experiments, in order to see whether it would not be possible to cure a patient already bitten. He carried on, therefore, a series of experiments which led to the discovery that if the process of inoculation be begun within five days of the bite in animals in which the incubation period was at least fourteen days, almost every animal bitten can be saved; and that even if the treatment be commenced at a longer interval after the bite a certain proportion of recoveries can be obtained. Thus the application of this method of treatment to the human subject was not tried until it had been proved in animals that such protection could be obtained, and that such protection would last for at least two years, and probably longer.

The chance of success in the human subject appears to be even greater than in the dog or rabbit, seeing that on account of the resistance offered by the human tissues to the virus the period of incubation is comparatively prolonged; very rarely, if ever, does an outbreak of the disease in man occur before an interval of at least fifteen days. The first symptoms usually appear in the fifth or sixth week, sometimes not until the third month; exceptionally the incubation period has lasted for a year. Thus there is an opportunity of obtaining immunity by beginning the process of vaccination soon after the bite has been inflicted, the protection being complete before the incubation period has passed. In his earlier experiments Pasteur injected on each succeeding day emulsions from a cord dried for one day less until cords dried five days were reached; but later he used those dried for only three days. This was the "simple" ten-day method. It was soon evident that although this method was efficacious where the wounds were not severe, and were confined to parts in which the nerve supply was not extensively interfered with, it was often quite inadequate in serious cases, as of wounds about the face, or of wounds inflicted by a mad wolf, the virus

of which is more active and the lesions made more severe than that of the rabid dog of the streets. In these latter cases the injections which, in the simple treatment, are spread over five days are made in three days; then, on the fourteenth day, a fresh series of injections, or, rather repetitions, is begun, which lasts until the twenty-first day. This is the "intensive method." In the technique of the treatment, which is the same in both methods, a small portion (about 1 cm.) of the desiccated cord is rubbed up thoroughly with about four or five times its bulk of bouillon until a complete emulsion is made; this is then injected by means of a syringe holding several cubic centimetres, first on one side of the hypochondriac region and then, the following day, on the other, and so on alternately, to avoid irritation. With the observance of thorough asepsis no local reaction to speak of takes place, nor are abscesses ever formed. The results of Pasteur's method of protective inoculation, as recorded in the reports issued in the *Annales de l'Institut Pasteur* and those of other antirabic institutes in Italy, Russia, Roumania, etc., are very favorable. Since 1886, when the treatment was first commenced at the Pasteur Institute in Paris, upward of 20,000 persons bitten by rabid, or presumably rabid, animals have received preventive inoculations, with a mortality of only 0.5 of 1 per cent. The mortality of those bitten on the face or head was 1.25 per cent. of those bitten on the hand; it was 0.75 of 1 per cent. of those bitten on other parts of the body, a little over 0.25 of 1 per cent. As a rule, only those persons are treated who have been bitten on the face or hand or whose clothes have been lacerated so that the virus has passed into the wounds. Ordinarily, a certificate from a physician or a veterinarian that the animal was rabid is required before vaccination; but if the animal cannot be found or the wounds are severe, vaccination is performed without it. Taking only the cases in which rabies has been confirmed in the animal by a veterinary surgeon, the mortality of the cases treated at the Pasteur Institute in Paris is only 0.6 per cent.—a proof, it would seem, of the trustworthiness of the statistics. In view of this fact there can no longer be any doubt of the value of Pasteur's antirabic treatment. It has been stated by some that the percentage of persons killed by the bites of rabid animals is inconsiderable; but according to the reliable statistics of Leblanc, from 1878 to 1883, out of 515 persons bitten in Paris, 83 died of hydrophobia, a mortality of 16 per cent.; most authorities place the mortality at a much higher figure. Extensive bites on the face and head are considered to be particularly dangerous; the mortality of these is said to be at least 80 per cent. The bites of wolves seem to be more fatal than the bites of dogs or other animals; the mortality of these, in spite of the most intensive treatment, is stated to be still 10 per cent., as against a previous mortality, without specific treatment, of 40 to 60 per cent. But even Pasteur's antirabic treatment appears to be unavailable when symptoms of the disease have manifested themselves. Our results in the New York Department of Health have been very encouraging.

Other methods of immunization against rabies have been proposed by different investigators. But all of these methods have proved on trial to be unsatisfactory and unreliable, beside being not devoid of danger. As early as 1889, Babes and Lepp conceived the idea that it might be possible by means of the blood to transmit conferred immunity against rabies from one animal to another; but although the success of these investigators was not great, Tizzoni and Schwartz, and later Tizzoni and Centanni, worked out a method of serum inoculation and protection in rabies which is worthy of attention. In this method not the rabic poison itself but the protective material formed is injected into the tissues. These observers showed that the serum of inoculated animals is capable of destroying the pathogenic power of the rabic virus—not only when mixed with it before injection, but when injected simultaneously or within twenty-four hours after the introduction of the virus into the body. This serum treatment of rabies is still in the experimental stage. We ourselves have had no experience with it, nor has it been adopted in Paris, or, so far as we know, in other places. It is quite possible that others will not obtain such good results as the authors of the treatment, or that it may not prove so efficacious in the treatment of man as it has been found to be in experimental work.

The Cauterization of Infected Wounds.—It is commonly believed that unless a cautery is used within an hour after infection by a suspected animal it is useless to apply it. This belief is held by physicians in general, and also, apparently, so far as the literature seen by me indicates, by those familiar with rabies. For this reason physicians when applying a cautery later than an hour after infection do so largely as a matter of form, for its moral effect on the patient, and so the application is not thorough, and in consequence not effectual. There is no evidence to show that the cautery is useless after an hour; no systematic investigations have been published, so far as we know, to prove the point one way or the other.

We know that the virus of rabies is not carried into the system by the blood, but through the nervous system. Dr. Follen Cabot carried out an extensive series of experiments in our laboratory upon guinea-pigs which showed: (1) That 91 per cent. of guinea-pigs can be prevented from developing rabies if the wounds be cauterized with chemically pure nitric acid at the end of twenty-four hours from the time of infection, probably a larger percentage if the cautery be used earlier. (2) That fuming nitric acid is more effectual than the actual cautery of pure nitrate of silver. (3) That some degree of benefit is derived from thoroughly opening and swabbing out an infected wound within twenty-four hours from the time of infection when no cautery is used. I believe that he demonstrated that in cases in which the Pasteur treatment cannot be applied great benefit may be derived from the correct use of cauterization, even twenty-four hours after infection, and that even in cases in which the Pasteur treatment can be given, an early cauterization will be of great assistance as a routine practice, and should be

very valuable, as the Pasteur treatment is frequently delayed several days, for obvious reasons, and does not always protect. In the case of small wounds all the treatment probably indicated will be thorough cauterization with nitric acid within twelve hours from the time of infection. Our experience in dealing with those bitten by rabid animals goes to show that physicians do not appreciate the value of thorough cauterization of the infected wounds.

Pasteur Treatment by Mail.—For several years we have made a practice of sending the treatment by mail when the patients could not go for treatment. The results have been good.

But far more important than any treatment, curative or preventive, for hydrophobia in man is the prevention of rabies in dogs, through which this disease is usually conveyed. Were all dogs under legislative control and the compulsory wearing of muzzles rigidly enforced where rabies prevails, hydrophobia would soon become an almost unknown disease. This fact has been amply demonstrated by the statistics of rabies in countries where such laws are now in force.

APPENDIX.

Aggressins.

A FURTHER contribution has recently been made to the problems of virulence and immunity in the form of the "aggressin theory" of Bail.[1] Apparently it grew out of an attempt to explain the so-called "phenomenon of Koch"—an observation made years ago by Koch—to the effect that tuberculous animals when inoculated intraperitoneally with a fresh culture of tubercle bacilli succumb quickly to an acute attack of the disease, the resulting exudate containing almost exclusively lymphocytes. Bail found that if tubercle bacilli, together with sterilized tuberculous exudate, were injected into healthy guinea-pigs, the animal died very suddenly—*i. e.*, in twenty-four hours or thereabouts. The exudate alone had no appreciable effect on the animal, while inoculation with tubercle bacilli alone produced death in a number of weeks. He therefore concludes that there is something in the exudate that allows the bacilli to become more aggressive, and hence has called this hypothetical substance "aggressin." He thinks it is an endotoxin liberated from the bacteria as a result of bacteriolysis and that it acts by paralyzing the polynuclear leukocyte, thereby preventing phagocytosis. Heating the exudate to 60° C. increases its aggressive properties rather than diminishes them and small doses act relatively more strongly than larger ones. These facts he explains by assuming the presence of two properties, one that prevents rapid death is thermolabile and acts feebly in small doses, and one that favors rapid death and is thermostabile. He assumes that in a tuberculous animal the tissues are saturated with the aggressin and when fluid collects in the body cavities, as it does on injection of tubercle bacilli, it contains large quantities of aggressin, which prevents migration of the polynuclear leukocytes, but not of the lymphocytes, and hence allows the bacilli to develop freely, producing acute symptoms. In the peritoneal cavity of the normal animal injected with tubercle bacilli, on the other hand, are large numbers of polynuclear leukocytes which engulf the bacilli, thus inhibiting their rapid development, there being here no aggressin to prevent phagocytosis.

This theory has been applied to a number of infections, including typhoid, cholera, dysentery, chicken cholera, pneumonia, and staphylococcus infections. In all similar results have been obtained as

[1] Wiener klin. Woch., 1905, No. 9. Ibid., 1905, Nos. 14, 16, 17. Berliner klin. Woch., 1905, No. 15. Zeit. f. Hyg., 1905, vol. i. No. 3. Arch. f. Hyg., vol. lii. pp. 272 and 411.

with tubercle bacilli. When exudates, produced by virulent cultures of these various organisms and properly sterilized, are injected with fresh cultures into an animal death occurs in much shorter time than when the organisms alone are injected.

Moreover, it has been possible to immunize animals against these various infections by repeated injections of the aggressin in the form of exudates. This results in the formation of an "antiaggressin," which opposes the action of the aggressin, thereby enabling the leukocytes to take up the bacteria and thus to protect the animal. This has been done in staphylococcus, dysentery, typhoid, cholera, pneumococcus, and chicken cholera infections in animals. In addition a very marked agglutinative property of the blood is acquired for the bacteria in the animals so immunized.

Experiments Devised by Ehrlich to Show the Nature of Hæmolysins.

In order to give the student an idea of the methods employed by Ehrlich in developing his doctrine of immunity, the classical series of experiments made to show the nature of hæmolysins are here reproduced.

Ehrlich asked himself two questions: (1) What relation does the hæmolytic serum or its two active components, immune body and complement, bear to the cell to be dissolved? (2) On what does the specificity of this hæmolytic process depend? He made his experiments with a hæmolytic serum that had been derived from a goat treated with the red cells of a sheep. This serum, therefore, was hæmolytic specifically for sheep blood cells—*i. e.*, it possessed increased solvent properties exclusively for sheep blood cells. Basing his reasoning on the side-chain theory, Ehrlich argued as follows: "If the hæmolysin is able to exert a specific solvent action on sheep blood cells, then either of its two factors, the immune body or the alexin (complement) of normal serum, must possess a specific affinity for these red cells." To show this he devised the following series of experiments:

Experiment 1.—Ehrlich and Morgenroth, as already said, experimented with a serum that was specifically hæmolytic for sheep blood cells. They made this inactive by heating to 55° C., so that then it contained only the substance sensibilatrice (immune body). Next they added a sufficient quantity of sheep red blood cells, and after a time centrifuged the mixture. They were now able to show that the red cells had combined with all the substance sensibilatrice, and that the supernatant clear liquid was free from the same. In order to prove that such was the case they proceeded thus: To some of the clear centrifuged fluid they added more sheep red cells; and, in order to reactivate the serum, a sufficient amount of alexin in the form of normal serum was also added. The red cells, however, did not dissolve—there was no substance sensibilatrice. The next point to prove was that this substance had actually combined with the red cells. The red

cells which had been separated by the centrifuge were mixed with a little normal salt solution after freeing them as much as possible from fluid. Then a little alexin in the form of normal serum was added. After remaining thus for two hours at 37° C. these cells had all dissolved.

In this experiment, therefore, the red cells had combined with all the substance sensibilatrice, entirely freeing the serum of the same. That the action was a chemical one, and not a mere absorption, was shown by the fact that red blood cells of other animals, rabbits or goats for example, exerted no combining power at all when used instead of the sheep cells in the above experiment. The union of these cells, moreover, is such a firm one that repeated washing of the cells with normal salt solution does not break it up.

The second important question solved by these authors was this: What relation does the alexin bear to the red cells? They studied this by means of a series of experiments similar to the preceding.

Experiment 2.—Sheep blood was mixed with normal—*i. e., not* hæmolytic goat serum. After a time the mixture was centrifuged and the two portions tested with the substance sensibilatrice to determine the presence of alexin. It was found that in this case the red cells acted quite differently. In direct contrast to their behavior toward the substance sensibilatrice in the first experiment, they now did not combine with even the smallest portion of alexin, and remained absolutely unchanged.

Experiment 3.—The third series of experiments was undertaken to show what relations existed between the blood cells on the one hand and the substance sensibilatrice and the alexin on the other, when both were present at the same time, and not, as in the other experiments, when they were present separately. This investigation was complicated by the fact that the specific immune serum very rapidly dissolves the red cells for which it is specific, and that any prolonged contact between the cells and the serum, in order to effect binding of the substance sensibilatrice, is out of the question. Ehrlich and Morgenroth found that at 0° C. no solution of the red cells by the hæmolytic serum takes place. They therefore mixed some of their specific hæmolytic serum with sheep blood cells, and kept this mixture at 0° to 3° C. for several hours. No solution took place. They now centrifuged and tested both the sedimented red cells and the clear supernatant serum. It was found that at the temperature 0° to 3° C. the red cells had combined with all of the substance sensibilatrice, but had left the alexin practically untouched.

It still remained to show the relation of these two substances to the red cells at higher temperatures. At 37° to 40° C., as already mentioned, hæmolysis occurs rapidly, beginning usually within fifteen minutes. It was possible, therefore, to leave the cells and serum in contact for not over ten minutes. Then the mixture was centrifuged as before. The sedimented blood cells mixed with normal salt solution showed hæmolysis of a moderate degree. The solution became complete when a little normal serum was added. The supernatant clear fluid separated

by the centrifuge did not dissolve sheep red cells. On the addition, however, of the substance sensibilatrice it dissolved them completely.

The addition of red cells in the experiments was always in the form of a 5 per cent. mixture or suspension in 0.85 per cent.—*i. e.*, isotonic, salt solution.

The significance of the last of the above-cited experiments is, according to Ehrlich, at once apparent. It is that the substance sensibilatrice possesses one combining group with an intense affinity (active even at 0° C.) for the red cell, and a second group possessing a weaker affinity (one requiring a higher temperature) for the alexin.

So-called Ultramicroscopic Examinations.

The apparatus constructed by Siedentopf and Zsigmondy[1] makes visible in colloidal solutions very minute particles, which heretofore could not be seen even with the highest magnifications. Particles as small as a few microns are thus rendered visible.

Fig. 164

Virulent diphtheria bacilli. Cultures two days old. Unstained. × 2400. (After Siebert.)

This increased power in microscopic analysis is made possible by focal lateral illumination of the object to be examined. The greater the difference between the refractive index of the particles colloidally dissolved and the fluid which surrounds them, the brighter will be the appearance of the particles, and, therefore, the more readily visible. The illumination for the purpose is most intense, and furnished by an electric arc lamp.

The microscopic field, as will be seen by the photogram herewith, is dark; the objects which refract the light show as brightly illuminated,

[1] Annalen der Physik, 4te Folge, Band 10.

sharply defined pictures, in which the black margin corresponds to the contour of the object. The illuminated portion is surrounded by a fine dark zone, this in turn by alternate bright and dark zones, in which the illumination rapidly decreases.

With a suitable apparatus both stained and unstained bacteriological specimens can be examined.

Variation in Susceptibility of Guinea-pigs to Diphtheria Toxin.

Smith has recently shown that a small percentage of guinea-pigs shows a marked resistance to the poisonous effect of diphtheria toxin. This refractory condition is inherited from the mother. This fact has to be considered in the testing of toxin and antitoxin.

Diplobacillus of Morax-Axenfeld.

This organism appears to be the exciting agent of a fairly frequent and peculiar infectious disease affecting only, so far as known, the human conjunctiva. The organism was discovered independently by Morax and Axenfeld in 1896. It is not pathogenic for animals. The usual clinical picture is that of a "blepharoconjunctivitis." The subjective symptoms are relatively slight.

Examination of the conjunctival secretion shows the characteristic bacilli. These are arranged mostly in twos, though long and short chains are also found. The bacilli average 2μ long and 1μ wide, though smaller diplobacilli are often seen, probably younger forms. The ends of the organisms are slightly rounded, and usually of the same thickness as the rest of the cell. The line of demarcation between the individuals is distinct. The bacilli are not stained by Gram's method and have no distinct capsules. The organism grows only at near the body temperature and best on solidified blood serum or serum agar. The medium must be alkaline.

Bacteria in Ice.

Water when it turns to ice destroys a considerable percentage of the vegetative forms of bacteria. Spores are not, as a rule, injured. Ice six months or more after freezing contains very few bacteria even when made from polluted water. Not more than 10 per cent. of typhoid bacilli survive freezing. At the end of one week not more than 1 per cent. remain alive. Not one in one thousand survive for one month, and none for more than six months. The colon group of bacilli are a little more hardy than the typhoid bacilli.

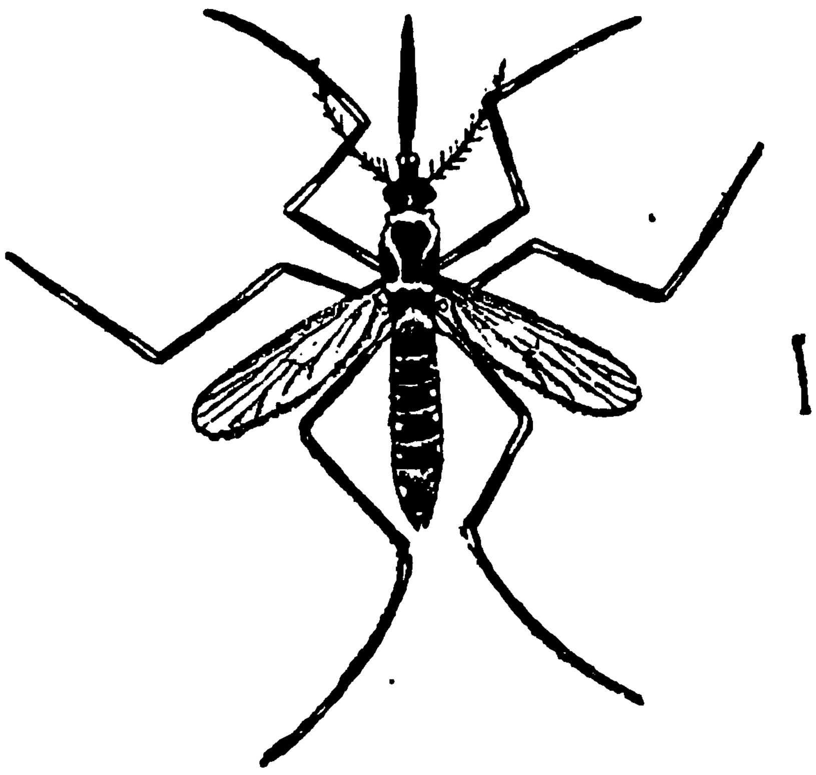
Stegomyia Fasciata and its Relation to Yellow Fever.[1]

The experiments made by Reed, Carroll, and Agramonte make it certain that yellow fever was transmitted by a mosquito (*Stegomyia*

[1] This account is taken from the article by Agramonte in Laboratory Work with Mosquitoes, by Berkeley.

fasciata), in the same way that malaria is transmitted by Anopheles. (See p. 442.) The name Stegomyia was suggested by the English entomologist Theobald, who separated this genus from the genus Culex, with which it was formerly classed. The salient characteristics of Stegomyia are: (1) the palpi in the male are as long, or nearly as long, as the proboscis; in the female the palpi are uniformly less than one-half as long; (2) the legs are destitute of erect scales; (3) the thorax is marked with lines of silvery scales. Stegomyia, or at least *Stegomyia fasciata*, is spread over a wide range of territory, embracing many varieties of climate and natural conditions. It has been found as far north as Charleston, S. C., and as far south as Rio de la Plata. There is no reason to believe that it may not be present at some time or other in any of the intermediate countries. In the United States specimens of *Stegomyia fasciata* have been captured in Georgia, Louisiana, South Carolina, and Eastern Texas. The island of Cuba is overrun with this insect. The fact that *Stegomyia fasciata* has been

Fig. 165



Adult female Stegomyia fasciata. (Drawn by Agramonte.)

known to exist at various times in Spain and other European countries may account for the spread of yellow fever which has occurred there once or twice in former times; the same may be said of the countries farther north in the United States, where *Stegomyia fasciata* has not yet been reported, but which have suffered from invasions of yellow fever.

Brackish water is unsuited for the development of Stegomyia larvæ. The species of *Stegomyia fasciata* seems to select any deposit of water which is comparatively clean. The defective drains along the eaves of tile roofs are a favorite breeding place in Havana and its suburbs; indoors they find an excellent medium in the water of cups of tin or china into which the legs of tables are usually thrust to protect the contents from the invasion of ants, a veritable pest in tropical countries. The same may be said of shallow traps, where the water is not frequently disturbed.

Like other *Culicidæ*, it prefers to lay at night. It is eminently a town insect, seldom breeding far outside of the city limits. Agramonte never

found *Stegomyia fasciata* resting under bushes, in open fields, or in the woods; this fact explains the well-founded opinion that yellow fever is a domiciliary infection.

The question of hibernation in the larval stage is important. Agramonte failed to get larvæ that could resist freezing temperature, and found that in the case of *Stegomyia fasciata* this degree of cold was invariably fatal.

The possibility of their being capable of life outside their natural element must also be considered from an epidemiological point of view. The dry season in the countries where this species seems to abound is never so prolonged as completely to dry up the usual breeding places. Experimentally, adult larvæ removed from the water and placed overnight upon moist filter paper could not be revived the following morning.

The question of the life period of the female insect is of the greatest importance when we come to consider the apparently long interval which at times has occurred between the stamping out of an epidemic of yellow fever and its new outbreak without introduction of new cases. The fact is that *Stegomyia fasciata* is a long-lived insect; one individual was kept by Agramonte in a jar through March and April into May for seventy-six days after hatching in the laboratory.

It was definitely shown by the experiment of the Army Medical Board upon non-immune persons that a period of at least twelve days at a temperature of about 83° F. was necessary before the infected insect could transmit the germ of yellow fever from the sick; later on a mosquito which reached the age of seventy days, in the hands of Dr. Carroll, was able to produce a case of yellow fever by stinging an American soldier fifty-seven days after it became infected.

These mosquitoes bite principally in the late afternoon, though they may be incited to take blood at any hour of the day. They are abundant from March to September, and even in November Agramonte was able to capture them at will in his office and laboratory.

The mosquito is generally believed to be incapable of long flights unless very materially assisted by the wind. At any rate, the close study of the spread of infection of yellow fever shows that the tendency is for it to remain restricted within very limited areas, and that whenever it has travelled far beyond this the means afforded (railway cars, vessels, etc.) have been other than the natural flight of the insect.

Experimentally, it has been found that the infected mosquito must be kept at about 80° to 85° F. for twelve days after having bitten a yellow fever patient. This is necessary in order to enable the parasites to perform the evolutions in the body of the mosquito that will render them capable of reproducing the disease. In winter insects kept at this temperature have failed to infect even after eighteen days.

Experiments have demonstrated that not all mosquitoes which bite a yellow-fever patient become infected, but that of several which bite at the same time some may fail either to get the parasite or to allow its later development in their body. This condition is similar to that seen in Anopheles, with regard to malaria.

How long do infected mosquitoes remain dangerous to the non-immune community? This question cannot be definitely answered at present; there is good presumptive evidence that the mosquito may harbor the parasite through the winter and be enabled to transmit in the spring an infection acquired in the fall. There is reason to believe that the mosquito, once infective, can transmit the disease at any time during the balance of its life.

With regard to the parasite itself, we cannot as yet say much. Numberless dissections of infected insects have been made, and serial stained sections have been prepared at various periods. The latter have led us to expect something tangible in the near future, though until now nothing definite has been discovered.

The question whether other genera of the *Culicidæ* or other species of Stegomyia are capable of transmitting yellow fever is still open for discussion. It is best not to forget that such a thing is possible, though the general opinion seems to be that yellow fever is restricted for its propagation to the genus Stegomyia.

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